Palynological Investigations of Selected Microfloras from the Cenozoic of Eurasia

By

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Palynological Investigations of Selected Microfloras from the Cenozoic of Eurasia

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بسم الله الرحمن الرحيم

In the name of Allah, the Most Merciful, the Most Kind

This humble effort is dedicated

With

Great love & honor

To

Esteemed and Exalted Messenger of Allah,

Our Holy Prophet

HAZRAT MUHAMMAD ﷺ

(Blessings and Peace Be Upon Him**)**

And his Family

The Eternal Source of Guidance for Humanity

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Shabir Ahmad

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Abstract

The present study was confined to investigate the selected palynomorphs from the Cenozoic of Eurasia using microscopic techniques to understand the climatic changes and vegetation's evolution. Sedimentary rock samples have been collected from Miocene Murree formation Islamabad, Murree, Kohat, Paleocene Patala formation Patala Nala within Salt range Punjab and Eocene Insect limestone Isle of Wights, United Kingdom. The samples were analyzed using the procedure of palynostratigraphic analyses and single grain method. Qualitative and quantitative features of the pollen were examined which helps in the identification of pollen. In this palynological study various samples were collected, analyzed, and then identified the palynomorphs using different previous and modern published literature. Botanical affinities and systematic description of the species were examined using light and scanning electron microscopy. Asteraceae was recorded as the dominant family and *Pinus* as a genus in the floral record of Miocene, Arecaceae in the Paleocene Patala formation Pakistan and Styraceae in insect limestone United Kingdom. Most of the angiosperms i.e Fabaceae and Poaceae have a poor fossil record but have an abundant palynological record in the study area. Palynomorphs are used as an investigative tool to examine the environmental fluctuations and their effects on the vegetations of a study area. By compiling the previously published literature of sporomorph data obtained from Eurasia compared with modern plants the palynoflora succession was developed. The current research work helps to find out the dominant vegetations within the Cenozoic (66 million years ago until today) of Eurasia. Most of the taxa studied here had a wide geographical occurrence in Eurasia and shows the abundance of angiosperms within this epoch. Highest polar diameter (75.75 µm), colpus length (34.5 µm) and colpus width (31 µm) were examined for the *Convolvulus*. This study provides evidence that in Paleogene times diverse rainforest vegetations were present in the Indian subcontinent and now restricted in some parts of the northern areas. Based on the palynoflora, the paleovegetation and vegetation units could be reconstructed for different time periods of the Cenozoic in the region of selected Formations along with paleoclimate in the potential recent analogues of the past vegetations. The study provides information about the vegetation's origin and environmental changes within the study area and to reconstruct it.

CHAPTER: 1

Introduction

1.1 Palynology and its significance

The word "palynology" comes from the Greek word "Palynin," which means "to scatter," and refers to the way that pollen is typically dispersed by animals, insects, wind, and other pollinators. Hyde and William (1945) coined the term "Palynology" to describe the study of pollen structures, their functions, dispersal, and preservation in specific environmental settings. Palynology is defined as "a study of pollen grains released by angiosperms, gymnosperms, and spores released by lower vascular plants (bryophytes and pteridophytes), according to Moore *et al*., (1991b). Pollen grains exist in the higher vascular plants, i.e., angiosperm and gymnosperm plants, while spores are the reproductive part in the lower vascular plants (bryophytes and pteridophytes). Pollen grains are similar to containers that carry the male gametes of gymnosperms and angiosperms. Although the two of these classifications, "pollen" and "spores," are separate, they both result from meiosis. They must disperse to carry out their role, but they can only do so if pollen from another member of the same species falls on the stigma and successfully fertilizes the egg and spores at a suitable location where they can germinate, flourish, and produce gametophytes. Their consideration within the same discipline of palynology resulted from the analogy that both require dispersion for successful function. Both of them have a well-sculpted, hard, robust, and resistant coating all around them (Moore *et al*., 1991b). Pollen and spores develop as a result of meiotic division, and both are quite different from each other. The pollen grains need to be dispersed to perform their roles if pollen grains fertilize the same species, and spores need suitable places for gamete production and germination.

1.2 Pollen Morphology

The walls of pollen grains are made up of two layers, exine and intine. The exine is made up of sporopollenin and a small amount of polysaccharides. Erdtman and Dunbar (1966) classified the exine layer of pollen grains into the sexine and nexine layers. Aperture is an important feature used for the identification of pollen grains. Colpi and pori are the two different types of apertures. Colpi are regarded as primitive features because of their pointy edges and boat-like appearance, pori are isodiametric. Pollen grains containing colpi are known as colpate, whereas pollen grains decorated with pori are known as porate. If pollen has both colpi and pori, it is referred to as colporate. These apertures are used to categorize the pollen grains into different groups.

They are divided according to number, position, and qualities. Prefixes to the type of aperture, such as porate, colpate, or colporate, are used to indicate the number of apertures.

Orientation of the aperture is other condition; if the apertures are evenly spaced over the surface, the name "poly" is used; otherwise, the term "zone" is used. Some terminology is uncommon, such as syncolpate pollen: Colpi are severely fused, for instance, in the myrtaceae. Another name for this substance is fenestrate (Faegri *et al*., 1964), sometimes known as echinolophate if a pollen has particular exine pattern with spiny ridges surrounding crests i.e pollen grains of *Taraxicum*, for example. *Acacia* taxa's pollen grains are grouped together into polyads, which are known as groups of grains. Mesocolpium or mesoporium are terms used to describe regions on the grain surface that are surrounded by colpi or pori borders, respectively. The portion of the pollen surface where no pori are present is known as an apocolpium or apoporium in cases of pollen when pori or colpi are in zono-arrangements. If the exine surrounding the aperture is inflated, it is referred to as bordered. The sexine portion of some Poaceae species swells and is known as an annulus. In some *Betula* species, the vestibulum is formed when two layers of exine split apart, creating a cavity around the pori. If these fissures form around zonocolporate grain fissures, they are known as fastigium. Similar to *Plantago* or certain other members of the Poaceae family, such as operculum, other grains have thickened portions of exine left in the center of the pori.

1.3 Pollen Sculpturing

Pollen wall sculpturing is the primary criteria in palynology for identifying pollen grains and is considered a desirable characteristic in taxonomy and systematics, particularly for families with eurypalynous traits. Intine (cellulosic) and exine (sporopollenin) are the two layers that make up the pollen wall (Scott, 1994). The sexine is made up of tiny rods known as columella if they support particular characteristics and bacula if they are free. These rods are called clavae if they are free and non-cylindrical; echinate if pointed; pila if they have swelling heads; gemmate if globular; verrucae if little warts; and scabrae if irregular lumps. The phrases tectate, intectate, and semi-tectate are typically used to describe sculpture. The grains are referred to as tectate, just if the heads of the columella are linked by tectum. The heads of the columella are linked on both sides in the cruciferae state, giving the appearance of a reticulum. The fine connecting walls are referred to as muri, and the cavity between them is referred to as lumina. The striate condition, where the lumen and reticulum run parallel and the structure in between is rugulate, results from little alteration of the reticulate condition (Moore *et al*., 1991a). The current study aims to increase knowledge of the distinctive sculpturing of pollen types, which could help produce an identifying key for future usage.

The pollen grain's exine and intine are each made up of various chemicals. The cell is shielded from the outside environment by the strong walls. Pollen has diverse sculpturing, walls, sizes, shapes, diameters, colpi, and pores in different families and flora (Mignot *et al*., 1994). Monocot pollen differs from dicot pollen, and angiosperm pollen differs from gymnosperm pollen. It plays a vital role in the protection of male gametes during pollination. Apertures play a key role in the identification of a taxon containing pori or colpi. Pollen adorned with pori is known as porated pollen, and pollen ornamented with colpi is known as colpate pollen. It may be mono, di, tri, tetra, etc. Pollen containing both pori and colpi is known as colporated pollen. Apart from these terms, some others are used poly, syn and zono are to be used (Faegri *et al*., 1964). Pollen sculpture also plays a vital role in species identification. Tectate, semi tectate and intectate are used for the sculpture of exine.

1.4 Palynology as a tool for the reconstruction of past flora and its environment

Numerous observational proxies (such as tree rings ice cores, corals, the stomata index, sporomorphs, stable isotopes, and charcoals) have been used to reconstruct paleoenvironment and paleo-vegetations. The current study is primarily concerned with fossil palynomorphs. Because of their exceptional resistance to chemical deterioration, widespread distribution, great productivity, and continuous records with vast numbers of specimens deposited in sediments, sporomorphs tells about the climatic variations and floral evolutions. Some researchers were able to reconstruct ancient vegetation and provide proof of previous floristic variety by comparing the similarities of paleoflora to present plant functional types (PFTs) using a public database (Jaramillo *et al*., 2006).

1.5 Significance of palynology in plant taxonomy

Pollen grains are an important feature of plant life. It plays an important role and provides helpful suggestions in the field of plant taxonomy (Moore *et al*., 1991b). Variation in morphological features of pollen helps in the taxonomic classification and delimitation. It gives hints to identify taxa below the family level. Palynology plays an important role in taxonomy for the classification of taxa up to the species level, offering an important system for classifying species at the genetic and family levels. It aids to solve the taxonomic problems for accurate identifications of highly related species (Erdtman *et al*., 1969). Pollen's morphological features can be used to classify and verify misidentified species. The study helps to identify evolutionary relationships, and place contentious taxa in the proper context (Nair & Hashimi, 1980), similar to Cronquist's classification of flowering plants based on pollen characteristics. Many taxonomists have used pollen morphology in the past to answer taxonomic problems (Wodehouse, 1935; Erdtman, 1966; Erdtman, 1986; Erdtman *et al*., 1961; Faegri & Iversen, 1964; Furness & Rudall, 2001; Erdtman, 1969; Erdtman & Dunbar, 1966; Tschudy & Scott, 1969). The objective of this study is to determine the vegetations origins and paleoenvironment within the study area.

1.6 Background Overview of Fossil Pollen

The biogeography of fossil pollen deals with the distribution of past pollen within geographic ranges. The study helps to understand and explore past environments and climates. It gives answers to many questions as to how different species evolved. The main questions in the present study are focused on plant taxonomy and biogeography. When did the first record of vegetation arise, and how does that compare to the contemporary plants to be identified in the study area? How has the biodiversity of species changed over time? What kinds of vegetation predominated in the study that was reported? What were the biogeographic ancestors of the current flora in Eurasia? How did these pollen types compare to the morph taxa found in nearby Cenozoic records? The following strategies were used to provide answers to all these queries: The morphological features of palynomorphs were determined using microscopic techniques in order to understand the vegetations origin within the Cenozoic of Eurasia. The palynological approach is taken into consideration in the current work to locate historical vegetation records in Eurasia. The rich floral diversity has yielded diverse pollen data. We have processed the rock sediments and investigated the pollen morphology of fossil plants along with their occurrences to answer these questions. Based on this data, we have identified pollen taxa and investigated how plant biodiversity changed in the study area. These results are vital to knowing how past vegetation's response to the climate has been and how to challenge it in the future.

The study of fossil palynomorphs and their distributions has been very important to learning about past vegetation and environmental records. It serves as a valuable indicator of past geographic conditions and aids in tracing the broad climatic fluctuations of previous geological ages. In addition, the diversity and abundance of fossils also help in defining the stratigraphy and environmental boundaries of rock units, particularly those where clearly distinct floral fossils are lacking. Apart from pollen grains, fossil remains are present in the form of algae, fungi, and wood fragments in many parts of the world, which have been investigated by various researchers. In the earliest pioneering researchers Rao and Vimal (1952) did work on the tertiary pollen of lignites from Palana (Eocene). Vimal (1952) investigated the record of palynomorphs from tertiary lignites in Punjab, Pakistan. Navale (1961) investigated fossil palynological records of fossil plants from south India. Ediger (1989) explored the algal and fungal palynomorphs from the Thrace basin, Turkey. Extensive field surveys were carried out at the different sites in the study area. It has been determined that study areas are very important to investigate fossil pollen and its correlations with the environment and geology. There has been relatively little published data on the Cenozoic era of Eurasia especially from Pakistan. The present investigation of the Miocene, Murree and Paeocene, Patala Formation is the first comprehensive palynological investigation of Pakistan's late Paleocene sediments.

The fossil palynomorphs were investigated using the procedure of Faegri *et al.* (1989), meaning the same individual pollen grains were examined using light and scanning electron microscopy. This increases taxonomic resolution and allows for a reliable affiliation to extant taxa (order, family, genus, and species). The specimens' size, shape, sculpting pattern, exine thickness, and aperture conditions were compared to those of Eurasia extinct and current flora. Based on the morphological features of both light and scanning electron microscopy, the specimens were identified (Halbritter *et al*. 2018). Previously, palynological studies were used for the identification of palynomorphs. Fossil pollen identifications were made from the reference collection of recent pollen and previous published literature and carried out up to the species, genera, and families. Image J and R software were used for morphological descriptions, and PCA was used for the analysis of paleoecological correlation (Grímsson *et al*. 2007). Based on the identification of animal fossil records, the age of selected formations was determined. In paleobotany, the field study was mostly conducted in the areas of mountains and river belts, where research work occurred very little in Pakistan. The main purpose of this research is to look at the palynomorph assemblages found in rocks and their correlation with the past environment. The morphological features of present palynological study were compared to the previous study. In addition, we made several changes to the initial matrix of morphological features in light of the research conducted by Jaramillo *et al*. (2014).

1.7 Cenozoic climatic variations in Eurasia

The Cenozoic climates of Eurasia were considered to be more humid and warmer than the current, having shallow latitudinal gradients (Jiménez-Moreno and Suc, 2007). This information was supported by the paleobotanical results of modeling studies (Micheels *et al*., 2011). The fossil plant record has frequently been used to reconstruct paleoenvironmental and paleoclimatic changes throughout the earth's history (Uhl *et al*. 2007). Analysis of fossil plant records in terms of environmental conditions, plant ecology, and taxonomy greatly influences the accuracy of such estimates. Floristic comparisons were frequently used to date vegetation reconstruction assemblies. The vegetation cover and paleoclimate of the Para Tethys-Tethys regions were more homogeneous in the Miocene than they are today, with warm climates and dense evergreen vegetation (Mai 1989). Palaeogeographic changes in the study area caused the evolution of Cenozoic floral evolution. In the Paleogene, the Arctic Ocean, Tethys, and Turgai Strait impacted the regional precipitation regime. Seasonal variations happened in the Paleogene due to the broad sea (Akhmetiev *et al*., 2012). The Cenozoic climatic changes of an area in a typical "East Coast Setting" are not yet fully documented quantitatively, despite the fact that these studies provide useful details. Also due to global climate changes, vegetation evolution occurred in the Cenozoic (Popova *et al*., 2013).

We still know very little about the Cenozoic climatic evolution on the Pacific side of Eurasia. In many areas of northern China, a cooling trend was observed at the Miocene level for paleoclimate studies. This study provides useful information, but there is no detailed record of Cenozoic paleoenvironmental changes in the "East Coast Setting" (Utescher, 2015). The evolution of the climate in eastern Eurasia, which is connected to the history of the East Asian Monsoon System, is further complicated by geological events including the uplift of the Tibetan Plateau and the opening of the Japan Sea back-arc. According to Sato *et al*. (2006), the tectonic activity of the Tibet Plateau, the Japan Sea back-arc opening, and Asia are all directly tied to the formation of the eastern Asian Monsoon system. In order to understand how historical continental climate anomalies have changed during the duration of the Cenozoic cooling and the processes that underpin them, it is helpful to compare the continental climate records from the two sides of the continent. The vegetation in Pakistan has Gondwanan, Eurasian, and Tethyan predecessors. The Gondwanan heritage is represented by Pakistan's Indus basin, which is in the east and south of the country.

The present research work provides important information about the plant systematics, biostratigraphy, and paleoclimate of the study area. From a paleobotanical point of view, the study area has not been explored, and this work is considered the first from the study area. The current investigations make associations with other fields, including evolution, plant ecology, genetics, molecular biology, biotechnology, biochemistry, and environmental biology. This study is very important for the reconstruction of palaeovegetations and paleoenvironments. The project's goal is to describe the palynological study of fossil pollen observed from selected Formations in Eurasia. The evolution of fossil plants from various ages is quantified using stratigraphy. The current study looked at certain prehistoric pollen records from different localities. Palynomorphs are important sources for determining an area's environmental fluctuations. These are useful research tools in a variety of subjects for studying the paleo-climatic conditions that affect vegetations in any place. Pakistan's stratigraphic committee has been working on the country's stratigraphic nomenclature. Paleoenvironmental changes in Asia happened primarily during the Cenozoic period. The above-mentioned literature emphasizes a wide range of geological or stratigraphic variations, demonstrating the tremendous diversity of vegetation over geological epochs. In this thesis, I explored the past vegetation record and how it interlinks with the environment and climate in the study area by studying the palynological investigations of microflora from the Cenozoic era of Eurasia. The study of fossilized pollen and spores at various locations has gained increasing importance over the last 70 years, resulting in the emergence of paleopalynology as an important paleobotany discipline. In this study, the Miocene Murree Formation, Paleocene Patala Formation, Pakistan, and Eocene Insect limestone were studied briefly as below.

1.8 Murree formation

The Miocene Epoch is of great importance for the evolution of plants and animals. It is further classified into 3 groups of lithology for palaeoenvironment and palaeoclimate changes, i.e., the late, middle, and early Miocene Epochs. Based on the proxy's data, different paleoclimatic conditions existed that were quite different from the present climate. Considering the neglect of the palynological data from the study area, we have investigated the paleoflora of the proposed era through pollen analysis from the Murree Formation. In this epoch, significant climatic and environmental variations occurred in many parts of the world, especially Asia (Sun & Wang, 2005). Pollen grains and spores present in rock sediments serve as proxies to indicate the previous vegetation and environment of an area. The knowledge of palaeoflora from sedimentary strata of the Miocene has produced a rich record of fossils, including animals and plants (Flynn & Jacobs, 1982; Shah, 1977), while most of the studies of Miocene palynomorphs from all over the world were reported based on light and scanning electron microscopy. In this paper, we have compiled the data from the sedimentologic, stratigraphic, and systematic points of view to know about the vegetation's evolution and depositional environment of the study area.

The Himalayas developed during the Tertiary Period due to collisions between the Eurasian and Indian plates. It is further divided into higher, lesser, and sub-Himalayan ranges, are thought to be the primary source of the Murree Formation. In terms of structural evolution, study area shares a strong tectonic affinity with the Himalayan orogeny. The study area has been given the name Murree after the Murree hills in the Rawalpindi area of Pakistan. The Miocene epoch was crucial to the evolution of plants and animals. Based on the data from the proxies, several paleoclimatic conditions existed that were substantially different from the current climate. We have analyzed the palaeoflora of the hypothesized age by pollen analysis from the Murree formation. The study of palaeoflora from Miocene sedimentary layers has yielded a rich record of fossils comprising animals and plants (Flynn & Jacobs, 1982), whereas most studies of Miocene palynomorphs from throughout the world have relied on light and scanning electron microscopy. In this work, we collected data from sedimentologic, stratigraphic, and systematic perspectives to learn about the Miocene vegetation development and depositional environment of Pakistan's Murree formation. A study provides systematic linkages about the existence of fossil floral records. Paleo-environmental analysis helps in determining the physical, biological, and chemical conditions of the environment. It helps in the identification of the microfossil plants and determines the estimated age of the rocks (Shah, 1977).

It is expanded to include the areas of Islamabad, Murree, Rawalpindi, Kohat, Potwar, and Kashmir. The strata are comparatively widely distributed in the Oligocene. The paleoenvironmental study of the Miocene was unexplored and had a great interest in determining the paleoenvironment of the study. It provides important information to understand how the Indo-Asian tectonic collision and the environmental change leading up to the Miocene-Eocene Transition are related to the fossil plant pollen record in the CMB (Strother *et al*. 2017; Yar *et al*. 2021).

The study is very important and interesting to palynologists in South Asia (particularly in India) and around the world. The sediments of the Murree formations were deposited (23.03-5.33 Ma) in the foredeep of the Margalla hills. It consists of dark-red, purple sandstones, intraformational conglomerates, reddish mudstones, purple gray-dark shales, and siltstones. The lower formation predominantly consists of mudstones, siltstones, and shale, while the upper Murree formation consists of arenaceous lithology. The Murree Formation was classified into 11 facies based on its sedimentological features. Near Ghori, between the Kuldana and Murree formations, there is a fossil record of foraminifera (Mughal *et al*., 2018). Islamabad, Kohat, Murree, and Attock are in the Murree Formation. The Murree Formation's sedimentary strata are made up of a variety of coarse to fine-grained units. The Murree Formation in the Attock district is made up of lamellated sandstones, claystones, and conglomerates. It has created a special fossil record of silicified wood, fish, frogs, and mammalian bones. (Shah, 1977). In Fatehjang, many mammalian bones have been recorded, i.e., *Hemimeryx* sp., *Brachyodus giganteu*s, and *Teleoceras fatehjangensis*. In Potwar and

Kohat, it reveals a fluvial environment, while in Kashmir-Hazara, it shows a shallow marine environment. In the Kashmir zone, dispersed forms of sediment were recorded from this formation (Singh *et al*., 2000). It has been further classified into two types: lower and upper Murree horizons. In the Siwalik sedimentary strata of the Darjeeling Himalaya, sections are exposed and enriched with fossil palynomorphs. For the eastern Himalaya, Siwalik Group, and India, a humid environment was investigated (More *et al*., 2016). Our study also shows river meandering depositional environments in the study area (Kundu *et al*., 2012). The Murree Formation in Kohat is made up of medium- to coarse-grained sandstones. It reaches its maximum thickness of around 3,030 m in northern Potwar, although it thins out to about 9 m thick in western Kohat. Numerous mammal bones, including those of various even-toed ungulates, rhinoceroses, and creatures resembling hippopotamuses, have been found in Fateh Jang. (Malkani & Mahmood, 2016). Based on the fossil mammalian record this formation has been restricted to the Miocene epoch (Shah, 1977).

1.9 Patala Formaion:

The "Patala Shale" was given the official name Patala Formation by the Stratigraphic Committee of Pakistan, and its use was spread to other regions of the Hazara provinces and Kohat-Potwar. The Patala Nala, in the salt range of Pakistan (Lat. 32° 40' N; Long. 710° 49' E), has been found within the range of this section. The Stratigraphic Committee of Pakistan has assigned the Patala formation for the Patala Nala section, but it is also found in the regions of Hazara and Kohat-Potwar (Davies & Pinfold, 1937). The shale is selenite-bearing, carbonaceous, and calcareous in some places, and it also contains marcasite nodules. The nodules are white-light gray limestone in texture. The Patala Nala section exposed in the Salt Range (Lat. 32° 40' N; Long. 710° 49' E) has been designated as the type section. The shale has selenite-bearing, carbonaceous, calcareous, and marcasite nodules. In the Patala Nala section, the formation is divided into three sub-divisions. The lower part is brown-gray to dark-gray shale with thin nodular limestones. Along with the shales, the formations also contain marcasite nodules in the calcareous and carbonaceous regions (Kazmi and Jan. 1997). The middle part consists of grayish shale with limestone, sandstone, and claystone beds. The upper part is represented by dark brown sand and limestones containing a large number of fossil planktonic records (smaller and larger foraminiferans) and other macrofossils. (Frederiksen, 1994) recorded palynological records of fossil plants from the Palaeocene Patala formation. Locally, there are economically valuable coal seams (Dandot area). In Kohat, it contains dark gray shales and light gray argillaceous limestone beds. The shale in Hazara has nodular limestone interbeds and is greenish brown to buff in hue. The geological formation of the Kala Chitta Range is made up of light brown and gray marl with sporadic interbeds of fine limestone. It is 27 meters thick at Khewra and 90 meters thick at Patala Nala. Smout and Haque (1956) collected bigger foraminifera from the Salt Range, including *Assilina dandotica*, *Kathina nammalensis*, *Daviesina intermedia*, *Actinosiphon tibetica*, *Discocyclina ranikotensis, Nummulites globules,* and *Operculina canalifera*.

The formation is considered one of the most important formations for economic aspects in the salt range of Pakistan (Kazmi and Jan, 1997). It has been exposed in the late Palaeocene age except for Hazara, which lies in the early Eocene age. Davies and Pinfold (1937) proposed the Patala Formation for the first time in the Patala Nala section. The sections are exposed within the tertiary rocks and are linked with Nammal Gorge in the southeast. Due to the rich sources of hydrocarbons in the salt range (coal, petroleum, and natural gas), the formation is economically and academically very important. The formation has been widely distributed in the areas of the salt range, northern Kohat, Surghar, Kala Chitta, and Potwar regions. Its thickness varies in different regions. It is 90 m thick at Patala Nala and 27 m thick at Khewra. In the Surghar Range, its thickness ranges from 30-75 meters; in the Kohat area, it ranges from 30-180 meters; in the Hazara area, it ranges from 60-182 meters; and in the Kala Chitta Range, it is 20 meters thick. In the salt range, the formation is composed of shales, limestones, marls, and limestones, which are nodular and white to grayish in color. In Kohat, the formation is mostly carbonaceous, containing dark gray shales.

The formation is represented in the Kohat area by dark gray shale that contains light gray argillaceous limestone beds and is occasionally carbonaceous. The shale in Hazara is greenish-brown to buff in color, with nodular limestone interbeds. The formation is represented by light brown and gray marl with thin interbeds of limestone in the Kala Chitta Range. It has been found in the Potwar area's subsurface and is widely exposed in the Kohat-Potwar and Hazara regions. It is 90 m thick at Patala Nala and 27 m thick at Khewra. The formation has been found in the Potwar area's subsurface and is widely exposed in the Kohat-Potwar and Hazara regions. The Patala Formation uniformly lies on top of the Lockhart Limestone throughout its entirety. It is enriched with fossil records containing foraminiferans, Ostracodes, and Molluscs. Smout and Haque (1956) recorded larger foraminifera, including O. patalensis*, D. ranikotensis, A. tibetica, A. dandotica, K. nammalensis, D. intermedia, L. conditi, O. canalifera* and *N. globules* etc from the Salt Range. In Hazara, smaller foraminifera were investigated by Latif (1970c) including *T. trigonula, R. trochiliformes, M. prehaimei, G. elongate* and *G. primitive*.

The coal from the Late Paleocene Salt Range is found in the Patala Formation. The Salt Range has the same formation throughout, but only the central and eastern parts of the range contain coal that is of any economic use. The center of the Salt Range contains many coal seams with thicknesses ranging from 10 cm to 1.5 m. The Potwar synclinoium's coal seams are typically thickest in the south, toward the anticlinal core, and thinnest in the north, toward the synclinal axis. Due to the deposition of coals in the Salt Range, scientists are interested in investigating its biostratigraphy and fossil records of plants and animals.

Palynofloras and macrofossils found in central Pakistan during Oligocene epo ch shows tropical wooded ecosystems with unique poaceae members (De Franceschi *et al*., 2008). For the early-late Miocene (18–11 Ma) of northwest India, forest vegetation has also been rebuilt using macrofossils and pollen; younger floras, however, show signs of drying out or becoming more seasonal. The study area has been considered the transitional zone due to the presence of nodular limestones in the lowermost subdivision. It showed that depositional environments were shallow marine waters. The formation declared environmental variations within the upper, middle, and lower parts, containing planktonic and benthic foraminifera. The formation declared environmental variations within the upper, middle, and lower parts, containing planktonic and benthic foraminifera.

1.10 Insect limestone

Insect limestone is considered an important assemblage for the preservation of fossil pollen, seeds, and fruits in the European Paleogene. It contains a very diverse insect fauna preserved in 3D (McCobb *et al*., 1998), which is presently monographed in the funded ITAS project started by Andrew Ross from the National Museum of Scotland, Edinburgh. Reid & Chandler (1926) first monographed this flora. In the basal portion of Bembridge, Solent Group, the flora is preserved within the fine grain. Hooker *et al*., (2007, 2009a) confirmed that the Bembridge Marls have the lowest 4.5 meters of have Eocene age and belong in subchron 1 of Chron C13r and not in Chron C13n by re-identifying the Eocene-Oligocene transitional interval magnetic chrorons (Gale *et al*., 2006).

The insect limestone flora has different components than the Messel oil shale. It shows similarities with the Messel oil shale flora due to the presence of plumed seed, winged fruit, and fruits with long awns. Similar to the Messel oil shale, insect limestone is abundant in insects, but it is almost devoid of vertebrate fossils. Yet, the presence of one feather attests to the fine-scale structural detail that the Limestone has managed to preserve. The insect limestone fruit and seed flora is dominated by aquatic and wetland plants, in contrast to the Messel oil shale, which is dominated by trees and lianas, such as numerous palms and a variety of Menispermaceae that are absent from this flora. The fruit and seed flora of the insect limestone similarly lacks large fruiting heads, illustrations of biological connections, and soft tissues. Between the Middle and Late Eocene, there was a temperature change that can be partially attributed to these variations, as well as different depositional environments and preservation techniques. Flora from this exceptionally preserved region, together with other important sites, provides important information about the origins of vegetation and the climates of the past. Climate change between the middle and late Eocene, as well as various depositional settings and preservation procedures, were both responsible for variations within the preserved flora. Insect limestone was formed at the end of the Eocene after severe cooling. In the study area, aquatic and wetland plants were dominant, while most trees and lianas i.e Menispermaceae were absent (Gale *et al*. 2006).

Konservat-Lagerstatten, in the study area, is renowned for the spiders and insects' superb preservation. Due to their terrestrial habitat, these arthropods are delicate creatures that only occasionally survive as fossils. The bug limestone consists of small-grained limestone that has the capability to preserve very tiny bodies. This limestone is also known as "opaque amber" because of its excellent three-dimensional preservation. It is located on the northern side of the island in an area called the Insect bed that is near the foot of the Bembridge Marls Member. Lakes and lagoons with freshwater or brackish water were where the Bembridge Marls were deposited (Daley 1999). Its collections were preserved in the Natural History Museum, London (NHMUK), containing fossil records of arthropods and plant remains. Insect limestone, known as the latest Eocene fossil, remains near the base of the Bembridge Marls Member. Preservation of species occurred in tabular to lenticellular bands in the form of small-grained micrite.

1.10.1 Sedimentology

The limestone particles are conchoidally fractured, tiny-grained argillaceous structures. Fresh parts have a blue-gray color and turn a brownish yellow. Coarser detrital layers that are up to 1 cm thick and planar silt laminae that are 1-4 mm thick are frequently seen in polished sections from Saint Helens, Thorness Bay, and Burnt Wood. Finely comminuted carbonaceous material is present in both laminae and layers; erosional bases and graded fining-up bedding are occasionally apparent. The strata are concretionary, not channel-infilling, as proposed by McCobb *et al*., (1998). and can be tracked laterally into the clay.

Early post-depositional formations that are representative of some sediment deformation include convolute lamination and flame bodies. Through the shear tension created by flow on the structure, flame formations occur when the soft muds are covered by the heavy particles and convolute laminations (Nichols, 1999). These structures and the frequency of the strata indicate the basin was a dynamic environment with regular influxes of material, even if there doesn't appear to be any pattern to the influxes. Mudstones, silts, calcareous marls, and clays with distinct concretionary bands comprised the insect bed. The series was accumulated in a cool environment, as evidenced by the tiny grain size and planar laminations. The limestone from St Helens, Thorness bay and burnt wood and St Helen having laminated clays contain halite pseudomorphs, indicating that the insect bed was likely accumulated in a highly saline environment.

1.10.2 Age of the Insect Bed

It was unclear whether the limestone insect bed is in the era of the early Oligocene or late Eocene (Insole *et al*., 1998). The insect bed dates back to the late Eocene to early Oligocene. The age of the transition in this epoch was between 33.9 million years, as surveyed by the stratigraphic chart 2012 at the international level. Due to climatic changes from warm to cool, it was considered an important time in the earth's history. Gale *et al*., (2007) made an effort to position the lithological units of the Solent Group within the chronostratigraphic time scale using magnetostratigraphy and other data. The uppermost 2 meters of the Bembridge limestone formation and the lowest 4 of the Bembridge Marls Member were recognized as the typical polarity zone. They designated Chron 13n as a kind and came to the conclusion that it belonged to the early Oligocene (Rupelian) period due to the existence of the Oligocene/Eocene period near the Bembridge Limestone Formation. Gale *et al*., (2007) positioned the lithological and stratigraphic units of the Solent Group using magnetostratigraphy and other data. The upper 2 meters of the Bembridge formation and the minimum 4 of the Bembridge Marls Member were recognized as the typical polarity zone. They designated this zone Chron 13n and came to the conclusion that it belonged to the early Oligocene (Rupelian) period because the Eocene/Oligocene boundary was located near the Bembridge Limestone Formation. The Eocene/Oligocene border is located either within Hamstead Member or Bembridge Member. Hooker *et al*. (2009) suggested that the Chron is basically subchron 1n of C13r. They use animal and microfossil biostratigraphy to support this claim, and hence, the Insect Bed is believed to be recent Eocene in age (Priabonian).

1.11. Justifications of the Present Study

The study area was explored for the first time to document the palynoflora of selected formations in Eurasia. The study was helpful in describing the palynomorph's morphology and depositional environments. Paleobotanical descriptions of fossil plants were used to examine the floristic affinities of the South Asian Eocene and Miocene flora. This would be very important and interesting to the palynologists in Asia, Europe and around the world. This record gives evidence about vegetation compositions and changes occurring in the proposed strata during different ancient periods, characteristics of present flora in papaleo-archives, and important conservation points of view.

Variability in family and genera wise plants based on elevational gradient helps in providing information about the past climate of the areas. The findings of the present study suggest the existence of higher vascular plants that would dominate the Paleogeneand Miocene strata. Further studies will be needed to understand its relationship with other strata and the origin of vegetation. It is critical to understand vegetation history in order to reconstruct historical floristic composition, climate, and environment.

1.12. Objectives of the study

The following goals are intended to be met by the project based on a palynological method for pollen analysis of fossil plants:

- Investigate fossil pollen and spores from sequential horizons of the selected Formations of Eurasia.
- Find out regional paleoclimate and climate changes in the deposition area during Cenozoic of Eurasia.
- To conclude about the paleophytogeographic history of the fossil pollen and its relation to the modern flora of Eurasia.
- Update the pollen profile by examining the morphological characteristics of fossil plant pollen.

CHAPTER: 2

Materials and Methods

2.1. Study areas: Cenozoic of Eurasia

Samples were collected from three different outcrops of Cenozoic ages because of their good accessibility and excellent exposures to investigate the microfossil record of the plants. The samples were taken from the Salt Range in the Patala Formation (Palaeocene age), Margalla hills, Siwalik ranges Kohat in the Murree Formation (Miocene age), Bembridge Marls Member, southern England (Eocene age). Lithological characteristics and composition of the sedimentary rocks, i.e., grain size, shape, color, and bed section thickness, were noted. The samples were processed for palynological investigations in the palynology laboratory, University of Vienna, Austria. Using scanning and electron microscopy, the detailed morphological characteristics of pollen were studied.

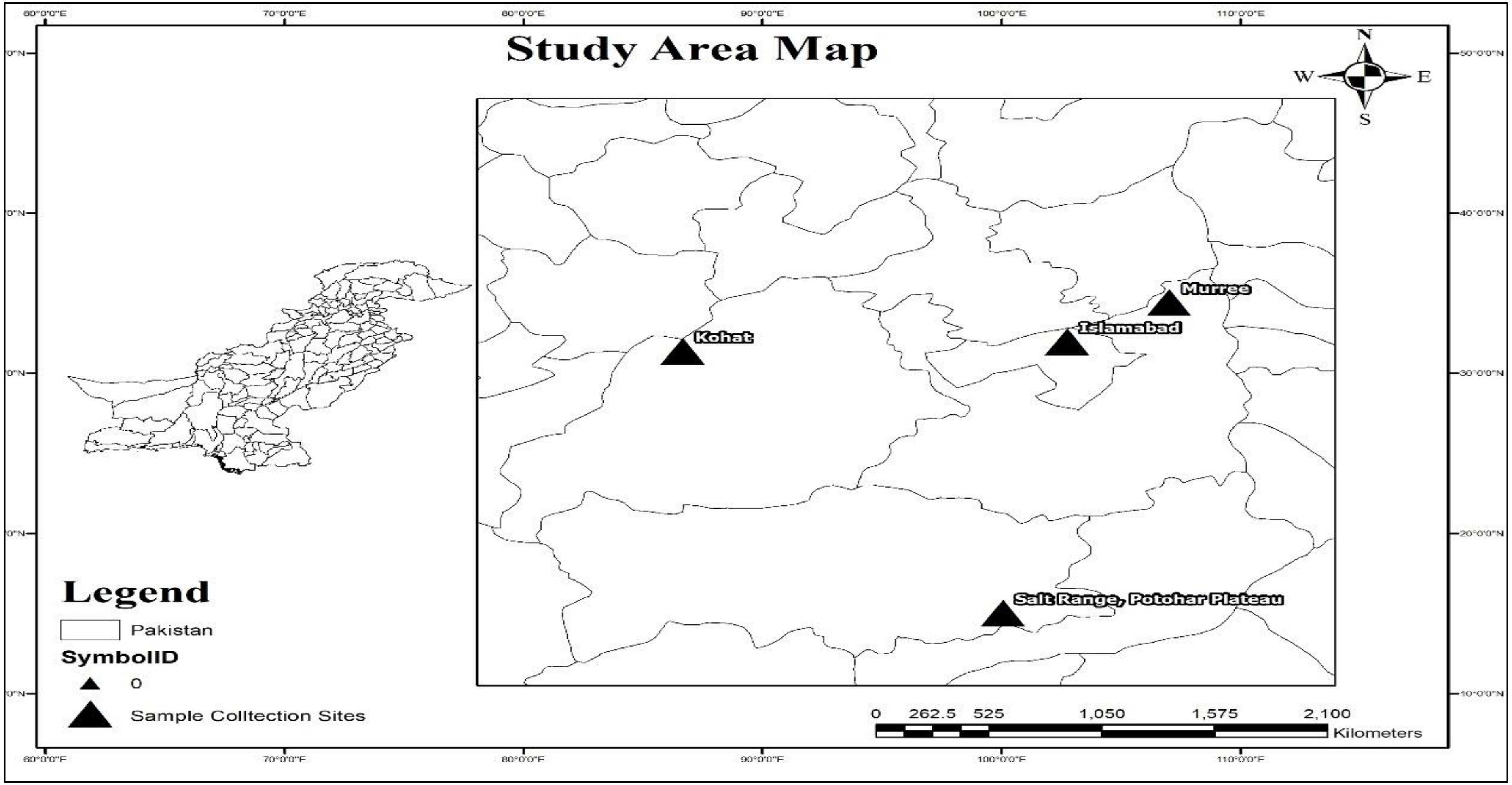

Figure 1. Map of the study area showing samples localities from Pakistan

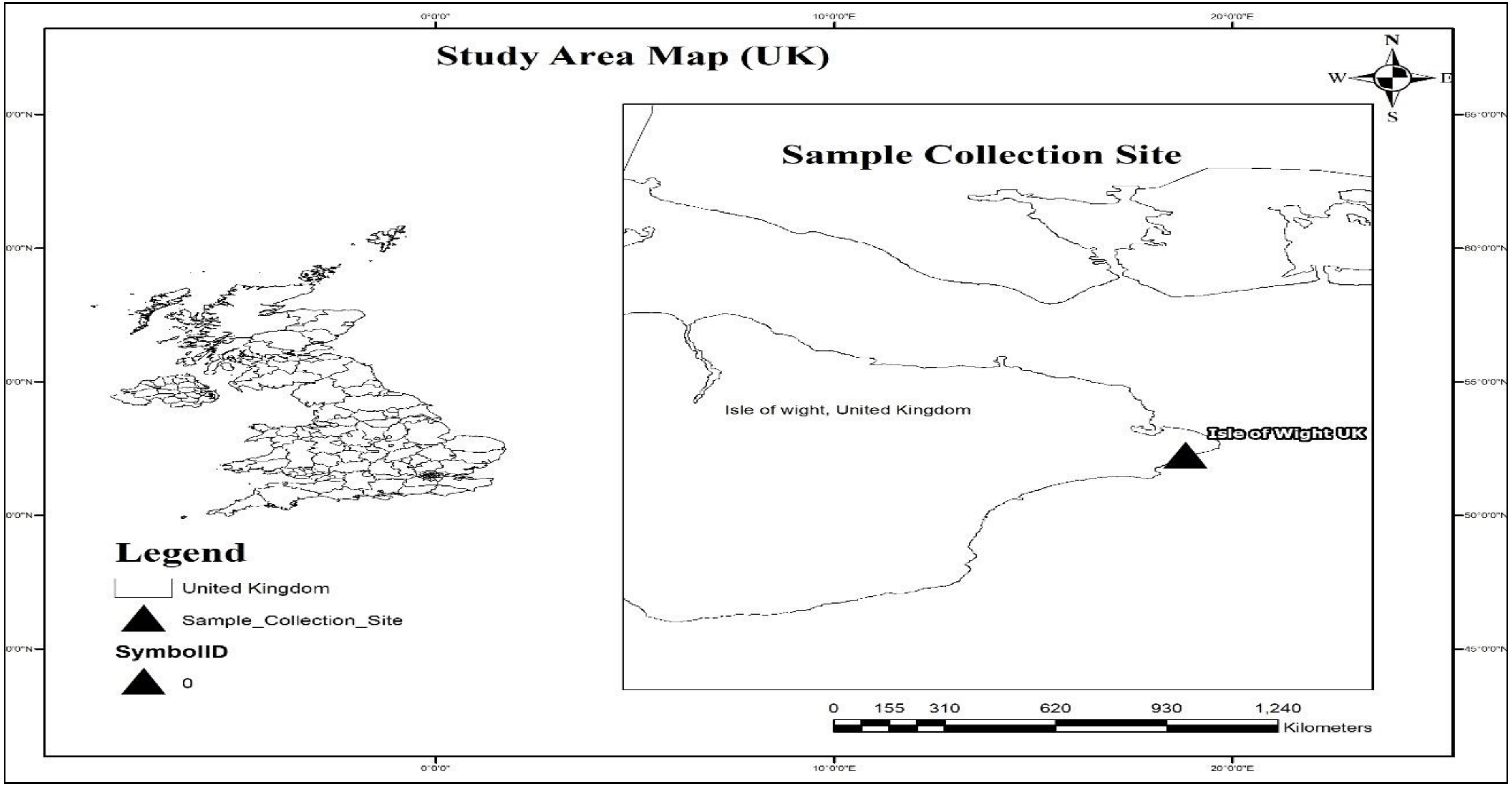

Figure 2. Map of the study area showing samples location from United Kingdom.

2.2. Samples collection

In field trips, the latitudes, longitudes, and temperatures of the study area were recorded during samples collection. Plant microfossils from the study area have rarely been investigated before, so this work was the first attempt to investigate the microfossils of the Cenozoic era. To ensure that the sediments contained palynomorphs, they were collected after mining sedimentary rock. Various samples were collected for paleopalynological purposes from shales and mudstones along different thicknesses of each formation, i.e., a 50-m thick Murree section. The samples were photographed, collected in polyethylene bags, and processed within the laboratory. Two different procedures were used for palynological analysis. In the first method, the samples were passed through the sieve cloth, while in the other method, they were not sieved, and the single-grain method was applied to them.

Plate 1. Panoramic view of Patala Nala, Salt range Pakistan

Plate 2. Panoramic view of Murree Formation Kohat, Pakistan.

Plate 3. Coal samples collected from Paleocene, Patala Formation

Plate 4. View of the collection site in Kohat, Murree Formation.

Plate 5. Collection of rock sediments from Islamabad, Murree Formation Pakistan

Plate 6. Collection of coal samples from Patala Nala, Salt range, Pakistan

2.3. Processing procedure for palynology

For sample preparations, simple techniques were used to understand that the palynomorphs would suffer less damage. Palynomorph assemblages were concentrated by a combination of preparation techniques, including crushing, demineralization (35% hydrochloric and 40% hydrofluoric acids), ultrasonic disintegration, and heavy liquid separation, and oxidation (with nitric acid and Schultze solution) to be ready for transmitted microscopy (Green, 2011).

2.3.1. Cleaning samples

To ensure that samples are free of dust particles i.e mud and soil, they must be cleaned by washing them under running tap water.

2.3.2. Crushing samples

Purpose of this phase is to create particles ranging in size from 1-2 mm to provide a large surface area for acid dissolution. As a precaution, a portion of the prepared sample was set aside for reference. The crushed sample is transferred to a clean polythene beaker and moistened with tap water to be ready for demineralization. In the present study, this step was found to be unnecessary because all of the analyzed samples were ditch-cut and washed throughout (Jones and Rowe 1999).

2.3.3. Number of samples required.

The number of samples required was determined by the lithology. The following sums have been adequate: 5–15-gram argillaceous sandstone, calcareous, or siliceous shale Sandstones weigh 25–30 grams, while argillaceous limestone weighs 35–50 grams and coal weighs 15-20 grams.

2.3.4. Testing for carbonates

This is done after the rock has been cleaned and is ready to be treated. In most cases, it is possible to tell from the color of the rock if it is composed of a significant amount of carbonate, but some prove difficult to evaluate and need to be tested. To test a rock, add a few drops of 10% HCL and see if the acid reacts. If there is a slight reaction, the rock may be dolomite, or it may contain a small amount of carbonate. If there is a vigorous reaction producing bubbles, then the rock contains a significant amount of carbonate. Dolomites need to be pre-heated, and rocks that exhibit an intermediate reaction level will also require the same treatment (Traverse, 2007).

2.3.5. Removal of carbonate material

To avoid secondary fluoride precipitation (CaF2, MgF2), $CaCO_3$ and $MgCO_3$ removed before HF treatment. To control any violent reaction, ethanol can be sprayed over the mixture. The samples were washed numerous times with tap water until a neutral PH was attained, then decanted from the water (Traverse, 2007).

2.3.6. Removal of the silicate material

40% HF (hydrofluoric acid) was carefully treated with the samples for the removal of silicates particles. This is accomplished by immersing the samples in HF for at least 24 hours and stirring at least every hour to speed up the reaction. To control any violent reaction, ethanol can be sprayed over the mixture. Following the completion of the digestion, the residues were washed many times to neutrality and decanted from water to prepare for the next processes (Traverse, 2007).

2.3.7. Concentration of organic matter

This can be obtained by washing the neutralized material under tap water through a 10 µm nylon mesh to remove fine residues.

2.3.8. Oxidation

To observe the color and kind of palynological organic matter (P.O.M.) prior to oxidation, one or two slides are made from the unoxidized section. The second half can be oxidized to eliminate unwanted components and to clean up the sample in general. When seen using a transmitted light microscope, this causes the color of the remaining organic material to lighten and become more visible. The residue is oxidized by adding nitric acid (HNO3) or Schultze solution (KCLO3: 3 HNO3). The reaction duration varies between 5 and 15 minutes, and the sample is constantly checked by pipetting and inspecting the residue under a microscope to avoid over oxidation. When the required oxidation level is reached, the material is passed through a 10 µm filter until it reaches neutral PH. A 2% KOH solution is then put to eliminate the oxidized humic acid. This step is unnecessary in the current investigation because the recorded palynomorphs have a lighter color and were easily described (Jones and Rowe 1999).

2.3.9. Heavy mineral separation

A solution of Zinc bromide (ZnBr) having specific gravity (1.9–2.0) larger than that of the organic matter was put to solution and centrifuged for 10 minutes. The suspended solution containing the palynomorphs was passed through 10 µm filter until its PH become neutral after which it is decanted from water and prepared for the next stage (Traverse, 2007).

2.3.10. Ultrasonic treatment

This process was utilized sparingly to break up organic matter clumps, notably in samples with lots of inorganic material (Green, 2011).

2.3.11. Slide preparation

The cleaned residue is mixed up with the dispersion solution P.V.A. (Polyvinyl alcohol solution). This material is pipetted onto a 32x22 mm cover slip and dried on a hot plate (25°C) beneath a dust cover. A clean glass slide (75x25 mm) was warmed (120°C), and small drops of Petropoxy-154 are smeared across its surface. The mixture is further heated to eliminate any air bubbles before being flipped and dropped onto the slide. It is critical to avoid the production of microscopic air bubbles. The cover slip and glass slide are both placed on the hot plate for 5–10 minutes. Each sample received one to two slides, and any remaining residue was preserved in little labeled tubes with stoppers and a drop of phenol as an antibacterial agent.

2.4. Sample preparation from Rock sediments

Different methods were used to extract fossil pollen from rock sediments. Some of these methods have been used by Traverse (2007), Moore *et al.*, (1991a), and Fægri and Iversen (1989). Most of these methods involve the sieving and final products of palynomorphs, with a focus on the numbers of pollen in the slide. The following paleopalynology method was used for the analysis of sedimentary rocks, with fewer variations: In this method, the solution is not sieved, due to which there are no greater chances of losing palynomorphs. The final mixture was kept in glycerine suspension in a small tube, and palynomorphs can be examined using a single grain method. This procedure has been able to study pollen morphological features using LM and SEM.

Sedimentary rock samples were collected for palynological studies from the shales. The sedimentary rock sediments were cleaned with the brush and then with water to remove the dust materials. The samples were ground with a pestle in a mortar. It was analyzed methods according to the method of (Grímsson *et al.*, 2016). The samples were reacted with hydrochloric acid (HCl) for calcium carbonate removal and hydrofluoric acid (HF) for silicate removal following the procedures of acetolysis and chlorination (Halbritter *et al.*, 2018). The pollen residues were shifted to glycerol, and using a single grain method, the pollen grains were extracted (Zetter, 1989). Pollen grains were photographed in both polar and equatorial views in light microscopy using an Olympus BX53 camera. Using nose hair fitted with needles, palynomorphs were transferred to the SEM stubs. The stubs were coated with gold and then studied through scanning electron microscopy (SEM) for detailed surface morphology.

Plate 7. Hammer placed on the rock for cutting the rock sediments.

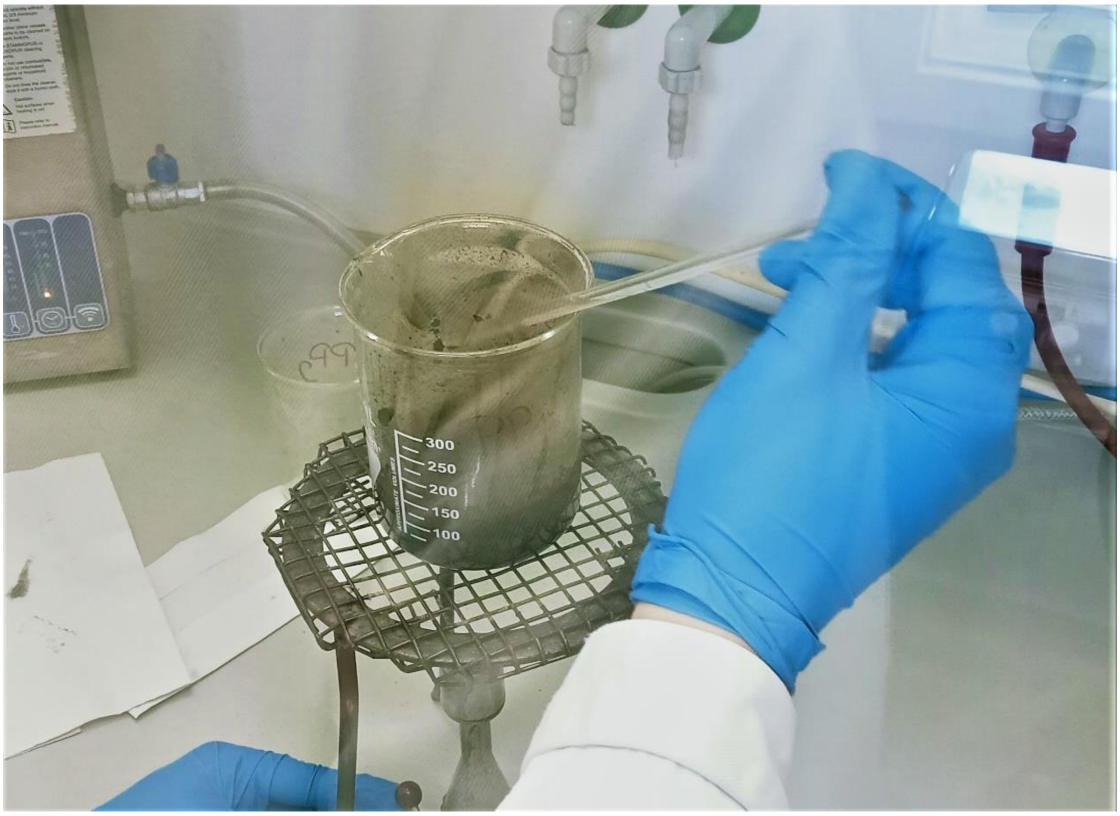

Plate 8. Rock sediments heated through spirit lamp along with acid treatment.

Plate 9. Centrifugation of the liquid samples to separate particles based on size.

Plate 10. Samples residues treated with bleaching powder to clean the palynomorphs

Plate 11. Samples were kept to settle down after ultrasonic treatment.

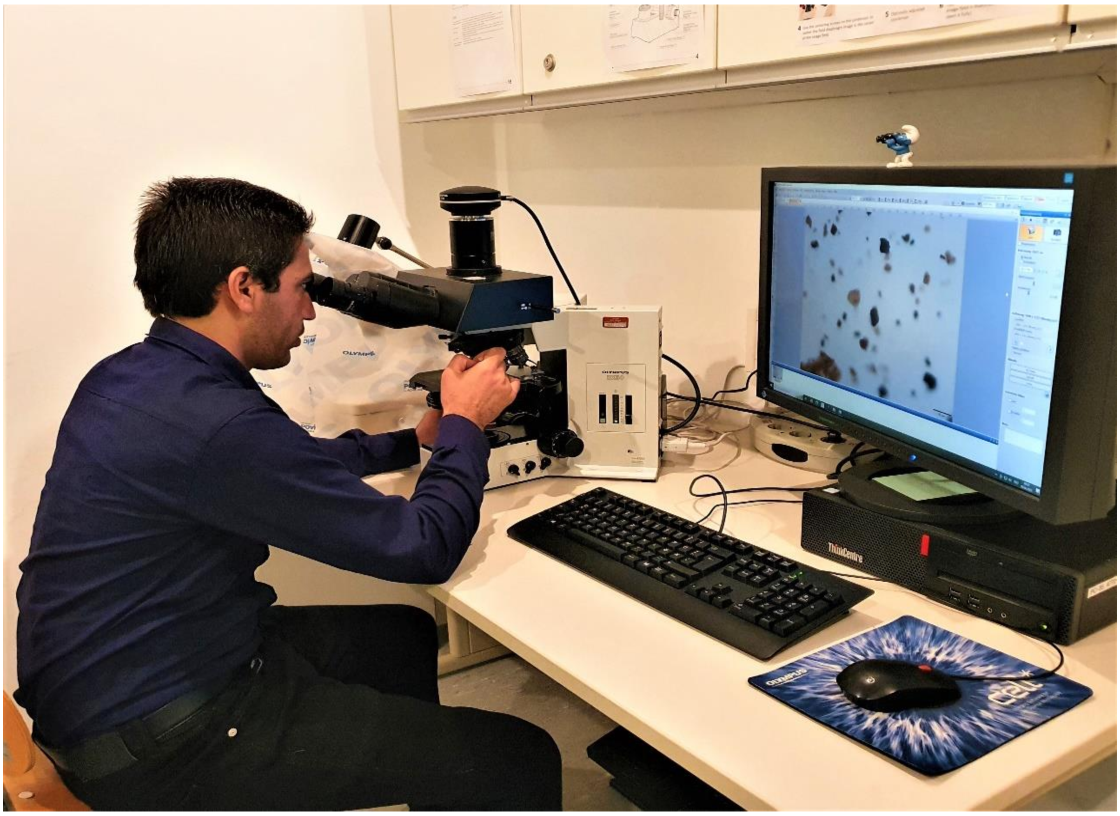

Plate 12. Photography of pollen with light microscopy using Olympus BX53 camera

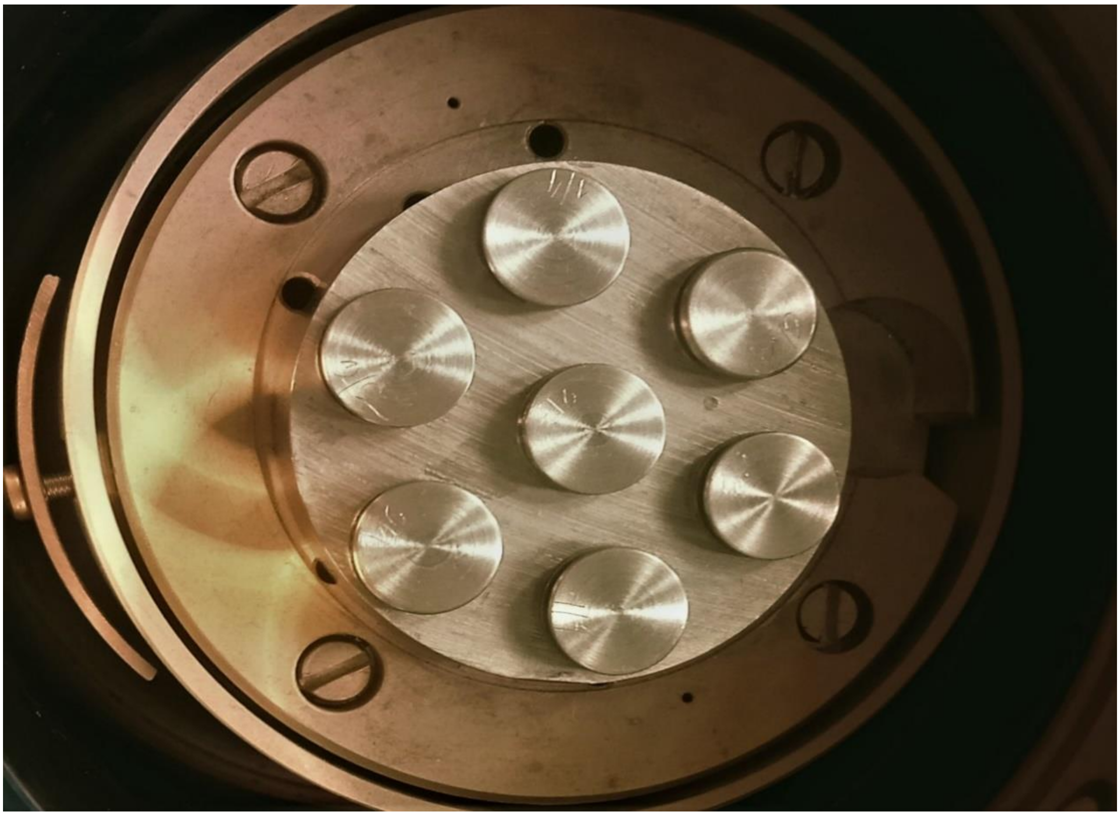

Plate 13. Pollen grains sputtered with gold foil in SEM sputter.

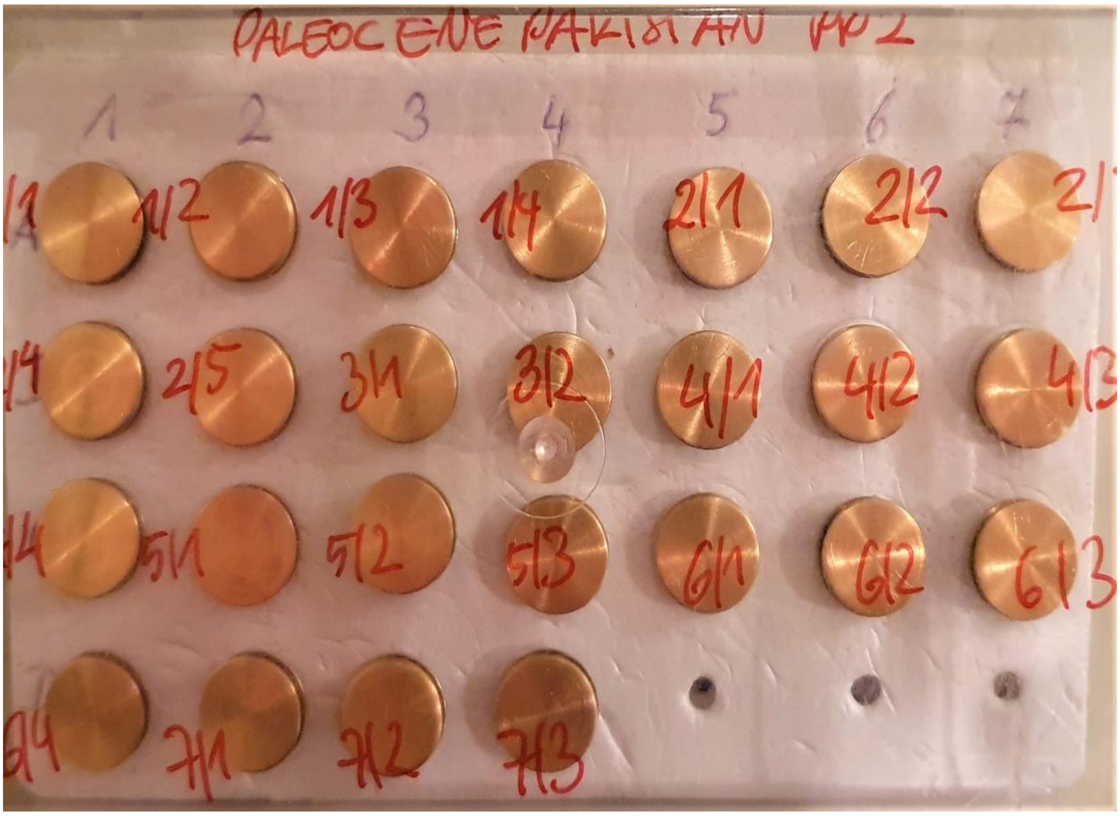

Plate 14. View of various SEM stubs of Paleocene, Patala Formation.

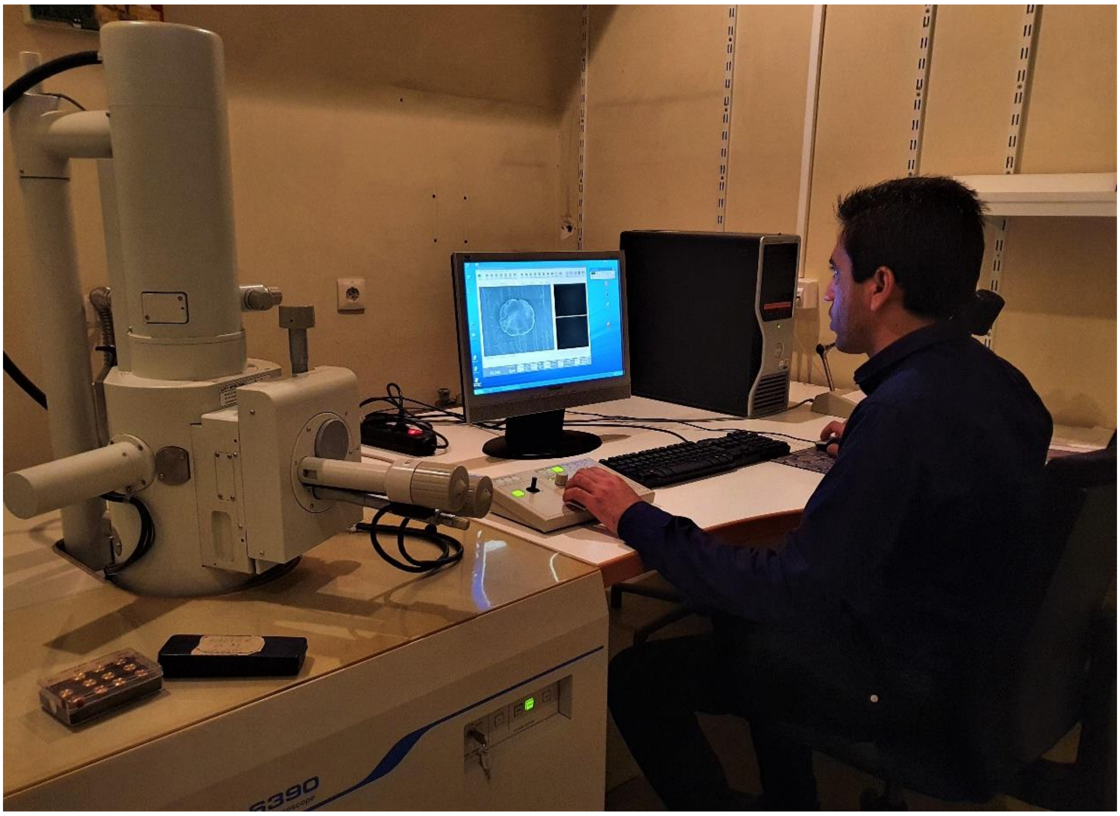

Plate 15. Photographs of pollen grains through scanning electron microscope

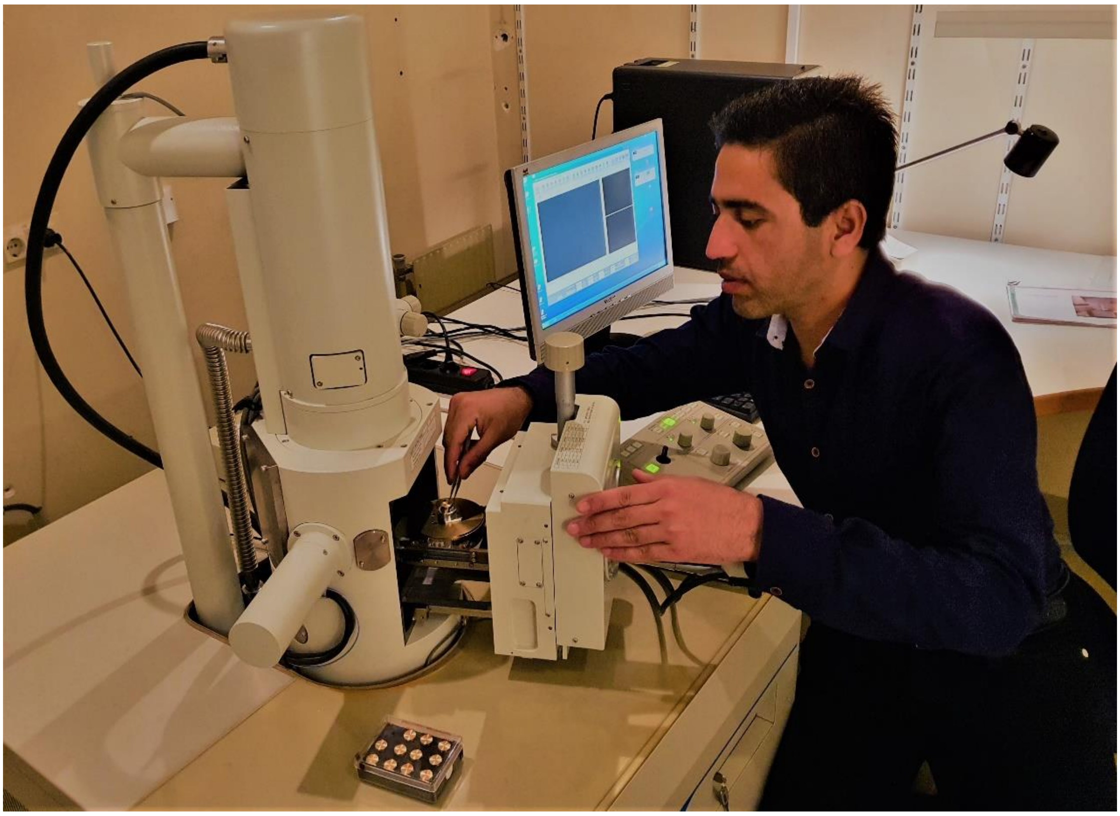

Plate 16. SEM stub taken out from the scanning electron microscope after photography.

2.5. Single grain method

Daghlian (1982) used the combine method for investigating the individual fossil grain, suggesting that the same grain can be studied using LM and SEM, and later, Zetter (1989) modified this method to use it more validly from a taxonomic point of view. The following equipment should be used to apply this method: a compound microscope, a needle with attached human nasal hair, glass pipettes, a glass slide, ethanol aboslute, a SEM sputter, and a scanning electron microscope. This method is very useful in that pollen grains are not sieved, and due to this, pollen grains have no chance to be lost, whether large or small pollen and the solution remains in small tubes. A compound microscope is used to study the pollen grains. Nasal hair fitted with iron metal is used to push or pull the pollen grains towards the glass slide. A new drop of glycerin was made on the other slide, and pollen grains were placed in it. Cover slips were not placed on the slide, so the pollen grains were turned, and photographs were taken in both polar and equatorial views with important pollen characters, i.e., sculpture, number of colpi and pores, etc. The SEM stub was placed under the stereoscope, and ethanol was also put in a small container. The pollen grains were picked up through the micromanipulator from the fresh glycerin drop and put on the SEM stub. During this time, small drops of ethanol were put on the SEM stub through pipettes to make the pollen grains free from glycerin. Up to 10 different types of pollen grains were put on the SEM stub, and further drops of ethanol were added for cleaning purposes. The stubs were coated with gold, and then pollen grains were photographed using scanning electron microscopy (Halbritter *et al.* 2018).

2.5.1. Making Glass Pipettes

It is vital to have enough glass pipettes for LM investigations. Glass pipettes were used to stir the sample to mix the particles well with each other. A small part of the samples was sucked through pipettes and transferred to the glass slide. The pipettes are dragged to the middle part of the slide so that they can produce a narrow strip of glycerin.

2.5.2. Producing a Micromanipulator

For making good micromanipulator to push pollen grains, a human nasal hair must be attached to the needle. Freshly collected human nasal hair was spread over a simple sheet. A teasing needle was taken and pushed into a 1-cm glue tube while turning the needle gently circularly. The needle was put out and pressed against one of the nasal hairs lying on the spread sheet. The distal end of the hair was attached to the distal end of the needle. The needle was placed within the small opening of the glass tube and pressed the tube gently while moving it circularly.

2.5.3. Applying the Single Grain Method

Glass pipettes were used to shake the sample and blow air to mix the particles with each other. Using glass pipettes, the sample was sucked out and transferred to the glass slide. The pipettes were touched along the glass slide and dragged in the middle to produce a narrow glycerin strip. The glass slides were placed within the working distance of the compound microscope and then pressed with a micromanipulator into the glycerin. Pollen grains from the interests are pushed to the edge of the glass slide, then picked with the nasal hair and transferred to another glass slide. A small drop of glycerin was made on the other slide, and nasal hair was dipped into the glycerin, and pollen was automatically detached. As no cover slip was used in this case, the pollen grains were turned back so that the polar and equatorial views could be photographed. With the help of nasal hair, the pollen grains were transferred into the SEM stub, where absolute alcohol was fixed so that it could clean the surface from glycerin. Up to 10 various pollen grains were put on each stub, and some more alcohol was added to make the surface of the pollen grains clear. The stub was coated with gold, and pollen grains were photographed using a scanning electron microscope (Halbritter *et al.* 2018).

Based on many morphological features, i.e., pollen size, shape, sculpture, number of colpi, and pores, the pollen grains are further classified into various groups. The supernatants were then retained after centrifugation. Samples were then put on a slide with the help of a camel brush and kept drying for a few minutes. Paraffin max was used for the preparation of permanent slides. In this study, qualitative and quantitative features of palynomorphs were studied, and photographs were taken through the camera Meiji Infinity 1 fixed with a Leica microscope.

Pollen grain size varies from species to genus and then to family level, but in genus it's similar to a great extent. Erdmann's (1966) protocols were used to study pollen shapes. Also, three different types of apertures were studied: colpate, porate, and colporate. The apertures of the pollen grains were prefixed according to their numbers, i.e., di, tri, tetra, penta, etc (Erdtman, 1986). If apertures look dispersed or scattered, then they are prefixed with panta, and they are considered zono if the apertures are oriented with symmetry. The different ornamentations of pollen grains are very helpful for the identification of taxa in the field of plant taxonomy (Erdtman, 1952).

2.5.4. Scanning electron microscopy

A nasal hair has been fixed with the dissecting needle, which was used to push the pollen grains towards the edges of the glycerin and then shifted to other slide for photography under light microscopy. The same grain can be photographed in multiple orientations because cover slip was not placed on the slide. The pollen grains were transferred to the SEM stub along with 100% ethanol which helps to remove the glycerin particles on the surface of pollen grains. The SEM stub was sputtered with gold for few minutes and then photographed the pollen grains with the scanning electron microscope (Halbritter *et al.* 2018).

2.5.5. Pollen Count and photography

Numerous palynomorphs were counted in each sample, apart from fungi, which were not to be considered. The Olympus BX51 microscope with an Olympus DP71 camera was used for pollen counts and photography. In the process of pollen counting, the cover slips were not fixed so that single pollen grains could be easily extracted and reoriented. Palynomorphs were firstly studied through LM and then transferred to the SEM stub for detailed morphology using JEOL JSM 6400 scanning electron microscopy. Extensive photography of the pollen grains was carried out. About 10 pollen grains were placed on each stub, coated with gold, and then kept within the scanning electron microscope. Based on different settings, positions, and contrasts, the photography was done until it achieved good results.

2.6. Taxa identifications

The palynomorphs were identified using pollen atlases, previously published literature, other collections, and a modern collection of plants (Xu *et al.* 2008). The distinguishing characters of pollen grains are very helpful for taxonomic identification in the field of plant taxonomy. Identifications of the taxa were carried out to the family, genera and in rare cases species level. For identifications of palynoflora, diagnostic features were taken from the published literature using microscopic techniques (Ahmad *et al*. 2023). The exine ornamentation of pollen grains is one of the most essential criteria. Pollen grains have been found to have different sculpturing patterns such as psilate, reticulate, and echinate. Furthermore, the presence of colpi, pori, and spines are crucial criteria for identification of species. Identification was done up to the family, genus, and, in some cases, species level. Light, scanning, and transmission electron microscopy have been used as standard tools for analyzing the pollen of prehistoric plants since the late 1970s. Since then, a massive amount of information on the morphological features of pollen from different plant evolutionary relationships has been accumulated. Numerous research articles are focused on one or more species. This gives us the knowledge necessary to palynological record in a palaeobotanical and paleobiogeographic context and allows us to correctly identify innumerable fossil pollen grains. The following distinguishing characters were studied in this research work:

i. Pollen size (polar axis, equatorial diameter, colpi length, colpi width, sulci length, sulci width, number of colpi and pori).
ii. Pollen shape (elliptical, circular, and quadrangular etc).
iii. Exine sculpture and aperture conditions.

CHAPTER: 3

Results and Discussion

3. Summary

In this paleopalynological study, microfloras were collected from the Miocene, Murree Formation Pakistan, Eocene, insect limestone United Kingdom, and Paleocene Patala Formation Pakistan. Using light and scanning electron microscopy, the diagnostic characters of palynomorphs belonging to diverse families were recorded (Figure 6). The pollen terminology was described using previously published literature. In this study, a total of 161 palynomorphs were analyzed and described briefly. Qualitative and quantitative features of palynomorphs of different sections were shown in tables and their micrographs in plates. For each section, figures were shown that provided a comparison among each species. The morphological characters of palynomorphs were examined, which are very important to study regarding paleoclimate, palaeoecology, and biogeography. The study provides useful information to understand the origin of vegetation and its link with climate change. Different parameters of palynomorphs, i.e., equatorial diameter, polar axis, colpi length, colpi width, sulci length, sulci width, columella height, description, and remarks, were presented for different species, which are very helpful for identifications of taxa in the field of paleobotany. In tables, pollen shapes, types, the presence of colpi/pores and exine sculpture were described. The study was conducted for the first time in the study area to highlight the distributions, identifications, and abundances of different floral paleopalynological records in the Cenozoic of Eurasia. Variability in family and genera-wise plants based on elevational gradient helps in providing information about the past climate of the areas. The findings of the present study suggest that angiosperms were dominant in the Cenozoic era. Lamiaceae species were dominant in the Miocene Murree Formation, Styraceae in the Eocene in the insect limestone, and Arecaceae in the Paleocene Patala Formation. Palaeobotanical records play an important role in the origin of Eurasia's modern flora with their correlation between vegetation and climate.

SECTION: I

Miocene, Murree Formation

3.1 Late Miocene Murree Formation

In the current palynological investigations, 89 different palynomorphs were investigated from 31 different samples, of which 48 were identified up to 12 different families. Quantitative and qualitative features of palynomorphs were discussed in Tables 1 and 2, and microphotographs were shown within the plates 1, 2 3 and 4. The findings of the current study demonstrated the value of palynological investigations in the field of paleobotany and demonstrated that the current research work is very useful for species classification. In this study about 60% of the palynomorphs in late Miocene were investigated. The study of palynomorphs in thick sections was challenging because identifications could be hampered by adverse cuts through the specimens and its dimensions, shapes, aperture morphologies, sculptural patterns, and exine thicknesses were compared to those of extant and current plants native to Eurasia. Based on morphological characteristics, the pollen grains were identified.

3.1.1 Proteaceae

The pollen grains of *Gevuina avellana* Molina were isopolar, radially symmetrical, triangular, sculpture psilate, pollen diameter 25.5 µm, colpi length 7.00 µm, colpi width 6.25 µm and exine thickness 2.25 µm (Table 2, Plate 17).

3.1.2 Rosaceae

In Rosaceae, unidentified genera and species were listed. Pollen grains were tricolporate, striated, suboblate, and micro-perforate, exine thickness of 4.00 µm and pollen diameter of 38.5 µm (Table 2, Plate 17).

3.1.3 Pinaceae

The pollen grains of *Pinus* were peroblate, subprolate, circular, dicolporate, angular, monoporate, psilated, and regulated, having a pollen diameter 55.75- 69.5 µm, exine thickness of 1.25–3.5 µm and colpi length of 17.25 µm (Table 2, Plate 17).

3.1.4 Typhaceae

Pollen grains of *Sparganium* were examined to be monoporate, circular, and psilated, with a pollen diameter of 38.00 µm and exine thickness of 2.75 µm (Table 2, Plate 17).

3.1.5 Fagaceae

Pollen grains were not identified up to genus and species level, tricolpate, prolate-spheroidal, inter-angular, regulated, psilated, pollen diameter 21.00–32.25, colpi width 6.25 µm, colpi length 7.00 and pollen diameter 21.00–32.25 µm (Table 2, Plate 17).

3.1.6 Cupressaceae

Interporopollenite's pollen grain was recorded to be psilate, inter-semilobate, psilate, pollen diameter 40.25 µm and exine thickness 1.25 µm (Table 2, Plate 17).

3.1.7 Myrtaceae

The family has an unidentified species and genus. Pollen grains were tricolpate, psilate, and angular, having an exine thickness 3.00 µm and pollen diameter of 23.00 µm (Table 2, Plate 17).

3.1.8 Asteraceae

Pollen grains of *Artemisia* were regulated, circular; exine thickness 1.5 µm and pollen diameter 22.5 µm. Pollen grains of Cichorioideae have unidentified species and genus which were echinated, spheroidal having and exine thickness 3.5 µm, pollen diameter 22.75 µm. The remaining pollen grains were tricorporate, prolate-spheroidal, spheroidal, having pollen diameter ranges 17.25–38.5 µm and exine thicknesses 3.25–4.00 µm (Table 2, Plate 17,18).

3.1.9. Chenopodiaceae

Pollen grains were identified up to the family level. It was spheroidal, pentaporate, regulated, pollen diameters 25.25 µm and exine thicknesses 3.5 µm (Table 2, Plate 18).

3.1.10 Liliaceae

Pollen grains were identified up to the family level. Its shape was peroblate, prolate, and exine sculpture psilate, with an exine thickness of 1.0-2.5 µm and pollen diameter 32.25-42.5 µm (Table 2, Plate 18).

3.1.11 Poaceae

Pollen grains were identified up to the family level. Exine sculpture psilate, monoporate, pollen diameter was 25.25 µm, exine thickness range from 1.75-2.25 µm, colpi width 6.25 µm and the colpi length was 7.00 µm (Table 2 & Plate 18, 19).

3.1.12 Lamiaceae

Pollen grains of Lamiaceae were examined to be circular, prolate-spheroidal, angular, semi-angular, and exine ornamentation psilate. The pollen diameter 30.25-43.00 µm, exine thickness 1.25–3.25 µm, colpi width 6.25 µm and colpi length 7.00 µm (Table 2, Plate 19, 20).

3.1.13 Discussion

In the current investigation, 48 species from 12 different families were identified and analyzed. Most of the pollen in this study is from the angiosperm families; Asteraceae, Cupressaceae, Liliaceae, Chenopodiaceae Lamiaceae, Poaceae, Fagaceae, Proteaceae, Typhaceae, Rosaceae and Pinaceae (Table 3). Using recent microscopic techniques, palynomorphs' qualitative and quantitative traits studied. Numerous morphologies of the palynomorphs, including suboblate, triangular, prolate, subprolate, round, spheroidal, and semi-angular, were examined. Additionally, psilate, reticulate, and echinate exine ornamented inter-subangular shapes were investigated. Similarly, many pollen types were identified, including dicolpate, tricolporate, monoporate, and pentaporate. Most of the species examined in this study had previously been documented in numerous other regions, including Africa, South Asia, Europe, and America. The current study's remarks on our understanding of the paleoflora of the Cenozoic sediments of Eurasia were significant (Khan *et al*. 2011). It’s one of the reliable techniques for reconstructing the palaeoenvironment and palaeoecology of the study area (Denk *et al*., 2021). The palynological record of Miocene strata is a more reliable indicator of the paleoclimate and environment.

The paleopalynological study of Miocene sediments was studied for the micro-fossil record (De Franceschi, 2008). In the present study, as compared to previous studies on *Quercus* pollen from China's deciduous forest, SEM results showed granulate, scabrate-verrucate, regulate, and rod-like features of exine ornamentations. The study of Cupressaceae, Asteraceae, Poaceae, Myrtaceae, Liliaceae, and Fabaceae in late Miocene sediments from Uttar Pradesh supports our findings for evidence of fossil pollen records and sheds light on the existence of minimal rain with a hot, humid climate in Southeast Asia (Paruya *et al*., 2017). The presence of *Quercus* in the study area suggests a warm, temperate climate, whereas *Pinus* suggests a temperate setting. Other ancient digs uncovered Rosaceae, Fagaceae, Lamiaceae, and Cupressaceae, which matched our findings. In the late Miocene-Pleistocene of China's Yunnan Plateau, mixed forests were recorded, mainly coniferous plants of the species Picea and Abies pollen (Wu *et al*. 2019). The early Miocene investigation in the Dulte Formation was conducted to examine the relationship between palynology and paleoecology. The palynological study of Miocene flora for Lamiales, Myrtales, and Bignoniaceae was examined using combined LM and SEM and revealed differences among different palynological characteristics that are relevant for taxon classification. The sea and tides had an impact on the formation, which suggests that flora thrived close to coastal areas like salt marshes or mangroves. The paleoenvironmental indication appears to be consistent with earlier records (Liu *et al*., 2020).

Pollen grains of the recent Asteraceae was shown to be tricolporate, spheroidal, echinated, and zonocolporated (Khan *et al*., 2018), while the pollen grains of Asteraceae examined in this study was spheroidal, prolate-spheroidal, and echinated ornamentations. Pollen morphological studies of present Poaceae members revealed monoporate-diporate, aerolate-scarbate tectum, but we observed primarily spheroidal, subprolate forms with inter-subangular and psilate ornamentations in our study (Usma *et al*. 2020). During the Miocene, climatic fluctuations were crucial for the creation and distribution of grasses. In contrast to earlier pollen records of Pinaceae in the area, which were monad, oblate, nearly circular, alveolate strucpture, and vesiculate-bisaccate, this study showed that the pollen grains of Pinaceae were bisaccate, circular-angular having psilate-regulate exine ornamentation (Khan *et al*. 2018). Pollen grains from *Quercus* had rod-shaped primary sculpturing units and were eutectate, prolate, sculpting perforate, microverrucate, and scabrate (SEM). Many *Ocimum* (Lamiaceae) species have been previously recorded in India and laterly spread to other parts of the world. The paleopalynological record of vegetation noted in previously published literature sources was primarily hexacolpate with reticulate exine ornamentations, as in the current study. The Miocene palynoflora of other Lamiaceae, which appear to have originated in China and spread to the southwest, is closely related to our findings (Yu *et al*. 2014). The *Sanguisobra minor* palaeoecological record from late Eocene–late Miocene has been recorded from Anatolia, and we can now show data on a greater distribution range than previously imagined (Akgün, 2021). The Cenozoic era of Eurasa is regarded as particularly vital due to significant climatic changes; however, the fossil plant records have only been studied on a few occasions.

The palynoflora of the Murree Formation in relation to Pakistan's palaeoenvironment was compared to the current flora using fossil data from depositional sites in China. The palynoflora of the Tibetan Plateau in the Miocene epoch was documented, briefly demonstrating climate changes and vegetation distribution, like our findings. Data from the genus *Pinus* indicated four unique pollen grains, but the species *Sanguisorba minor* emerged as the main pollen type in our investigation with two distinct pollen grains. With 12 species ascribed to the base of pollen grains, Lamiaceae is considered as the dominant family in study area followed by Asteraceae (9), Poaceae (4), and Liliaceae (2) while the remaining each family contain one pollen grain. The study revealed that *Ocimum basilicum* has an exine thickness that can reach a maximum of 4.25 µm. *Pinus* had the largest average pollen diameter at 69.5 µm, and Asteraceae pollen grains had the smallest average pollen diameter at 17.25 µm. The Asteraceae fossil pollen grains from Patagonia's Miocene Epoch were tricolporated and echinated in nature, whereas recent Asteraceae pollen possessed thick, spherical, tricolpate, and echinate exine ornamentations. The *Gevuina Avellana* (Proteaceae), as the earliest species, has tricolpated pollen, radially symmetrical pollen, and psilate exine ornamentation (Paruya *et al*., 2017).

The Miocene paleoflora in the study area gives knowledge for understanding paleoenvironmental variations. Paleopalynological study of the fossil pollen have been neglected in Pakistan despite being crucial to ecology and stratigraphy. To fully comprehend the paleoclimatic history of this region, additional research is required to integrate results from other proxies, including plants and isotopic data. It is very important for the Indian sub-continent paleobotany, paleoenvironment, and biogeography to be first in the examined area. Additionally, it facilitates the understanding of the origins of vegetation, the effects of long-term climatic changes, and the resolution of taxonomic challenges (Florenzano, 2019).

Table 1. Micromorphological characteristics of pollen—qualitative from late Miocene, Murree formation, Pakistan

S. No	Species/Taxon/Family	Pollen shape	Pollen type	Colpi/Pore	Ornamentations	Spines
1	*Gevuina Avellana* Molina	Triangular	Tricolporate	P	Psilate	A
2	*Sanguisorba minor* Scop	Circular	-	A	Psilate	A
3		Spheroidal	Tricolpaorte	P	Psilate	A
4	*Pinus* spp.	Circular	Monoporate	A	Regulate	A
5		Subprolate	Dicolporate	P	Psilate	A
6		Peroblate	-	P	Psilate	A
7		Angular	Monoporate	A	Regulate	A
8	*Sparganium* spp.	Circular	Monoporate	P	Psilate	A
9	*Quercus* spp.	Inter-angular	Tricolpate	P	Regulate	A
10		Prolate-spheroidal	-	A	Psilate	A
11		Prolate	-	A	Psilate	A
12		Prolate-spheroidal	-	A	Regulate	A
13	*Juniperous* spp.	Prolate	-	A	Psilate	A
14	Myrtaceae	Angular	Tricolpate	P	Psilate	A
15	*Artemesia* spp.	Spheroidal	-	A	Regulate	A
16	*Interporopollenites* spp	Inter-semilobate		A	Psilate	A
17	Chenopodiaceae	Spheroidal	Pentaporate	P	Regulate	A
18	Cupressaceae	Spheroidal	-	A	Psilate	A
19	Liliaceae	Prolate	-	A	Psilate	A
20		Peroblate	-	A	Psilate	A
21	Cichoriodeae	Spheroidal	Polyporate	P	Echinate	A
22	Asteraceae	Spheroidal	-	A	Echinate	P
23		Spheroidal	-	A	Echinate	P
24		Spheroidal	-	A	Echinate	P
25		Prolate-spheroidal	Tricolporate	P	Echinate	P
26		Circular	-	A	Echinate	P

27		Spheroidal	-	A	Echinate	P
28		Circular	Tricolporate	P	Echinate	P
29	Poaceae	Elliptic	Monoporate	P	Psilate	A
30		Spheroidal	-	A	Psilate	A
31		Prolate	-	A	Psilate	A
32		Subprolate	-	A	Psilate	A
33		Spheroidal	-	A	Psilate	A
34		Inter-subangular	-	A	Psilate	A
35		Circular	-	A	Psilate	A
36		Prolate-spheroidal	-	A	Psilate	A
37	Lamiaceae	Prolate-spheroidal	-	A	-	A
38		Semi-angular	-	A	Psilate	A
39		Circular	-	A	-	A
40		Circular	-	A	-	A
41		Angular	-	A	Psilate	A
42		Circular	-	A	-	A
43		Angular	-	A	-	A
44		Circular-lobed	-	A	Psilate	A
45		Prolate	-	A	-	A
46		Circular-lobed	-	A	Psilate	A
47		Circular	-	A	Psilate	A
48	*Ocimum basilicum* L.	Oblate-spheroidal	Hexacolporate	P	Reticulate	A

Table. 2 Quantitative features of fossil plants pollen from late Miocene, Murree formation, Pakistan

S. No	Species name	Exine thickness (µm)	Pollen diameter (µm)	Colpi length (µm)	Colpi width (µm)
1	*Gevuina Avellana* Molina	2.25	25.5	7.0	6.25
2	*Sanguisorba minor* Scop	3.25	21.75	10.75	2.25
3		4.00	38.5	-	-
4	*Pinus* spp.	2.5	55.75	-	-
5		3.75	59.00	13.00	17.25
6		3.5	69.5	-	-
7		1.25	53.5	-	-
8	*Sparganium* spp.	2.75	38.00	-	-
9	*Quercus* spp.	1.6	32.25	2	
10		3.25	21.00	-	-
11		1.25	29.25	-	-
12		2.00	51.25	-	-
13	*Juniperous* spp.	3.5	56.5	-	-
14	Myrtaceae	4.07	31.8	-	-
15	*Artemesia* spp.	1.5	22.5	-	-
16	*Interporopollenites* spp.	1.25	40.25	2.5	3
17	Chenopodiaceae	3.5	25.25	-	-
18	Cupressaceae	3.25	26.00	-	-
19	Liliaceae	1.00	42.5	-	-
20		2.5	32.25	-	-
21	Cichoriodeae:	3.5	22.75	-	-
22	Asteraceae	4.00	17.5	-	-
23		3.75	17.25	-	-
24		3.75	22.00	-	-
25		3.5	38.5	-	-
26		3.5	36.75	-	-
27		3.25	32.00	-	-

28		3.75	21.00	-	-
29	Poaceae	2.25	31.00	-	-
32		2.00	27.5	-	-
31		1.75	23.00	-	-
32		2.00	26.25	-	-
33		1.25	40.00	-	-
34		2.25	25.25	-	-
35		1.75	31.75	-	-
36		1.25	16.5	-	-
37	Lamiaceae	1.25	31.00	-	-
38		3.00	30.25	-	-
39		3.25	39.00	-	-
40		2.5	38.25	-	-
41		2.5	40.25	-	-
42		3.00	38.25	-	-
43		2.25	31.25	-	-
44		2.25	37.5	4.75	5.25
45		2.25	38.5	-	-
46		2.75	32.75	-	-
47		3.00	43.00	-	-
48	*Ocimum basilicum* L.	4.25	57.23	22.6	4.22

Table 3. Pollen category, families, taxa, and its counts

S. No	Category	Family	Taxa/Tribe	Counts
1	Angiosperm	Proteaceae	*Gevuina Avellana* Molina	1
2	Angiosperm	Rosaceae	*Sanguisorba minor* Scop	2
3	Gymnosperm	Pinaceae	*Pinus* spp.	4
4	Angiosperm	Typhaceae	*Sparganium* spp.	1
5	Angiosperm	Fagaceae	*Quercus* spp.	4
6	Angiosperm	Asteraceae	*Artemesia* spp	1
7	Gymnosperm	Cupressaceae	*Juniperous* spp *Interporopollenites* spp -	1 1 1
8	Angiosperm	Lamiaceae	-	12
9	Angiosperm	Chenopodiaceae	-	1
10	Angiosperm	Asteraceae	-	7
11	Angiosperm	Poaceae	-	8
12	Angiosperm	Liliaceae	-	2
13	Angiosperm	Asteraceae	Cichoriodeae	1
14	Angiosperm	Myrtaceae	-	1

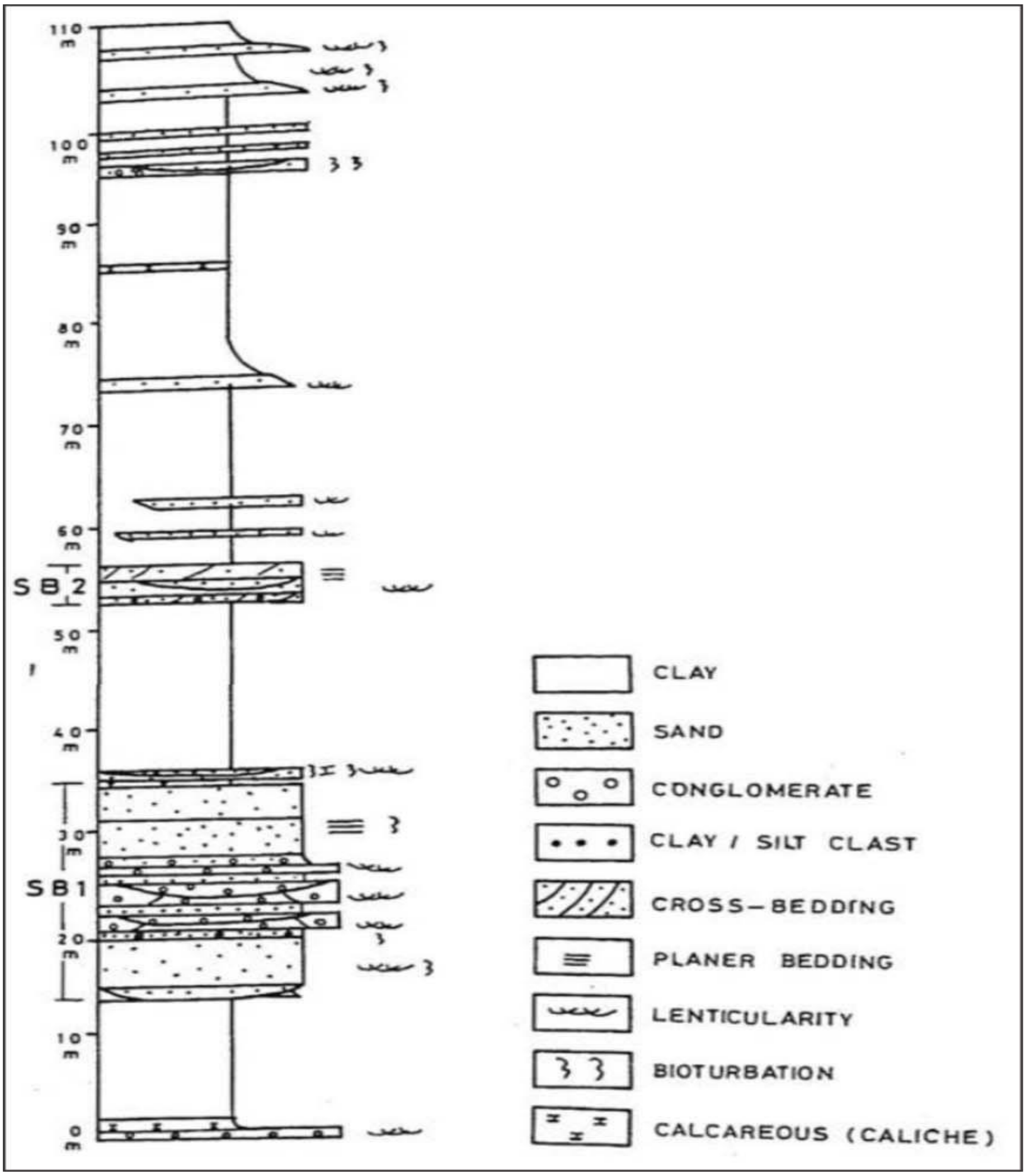

Figure 3. Stratigraphic column of the Murree Formation, Pakistan

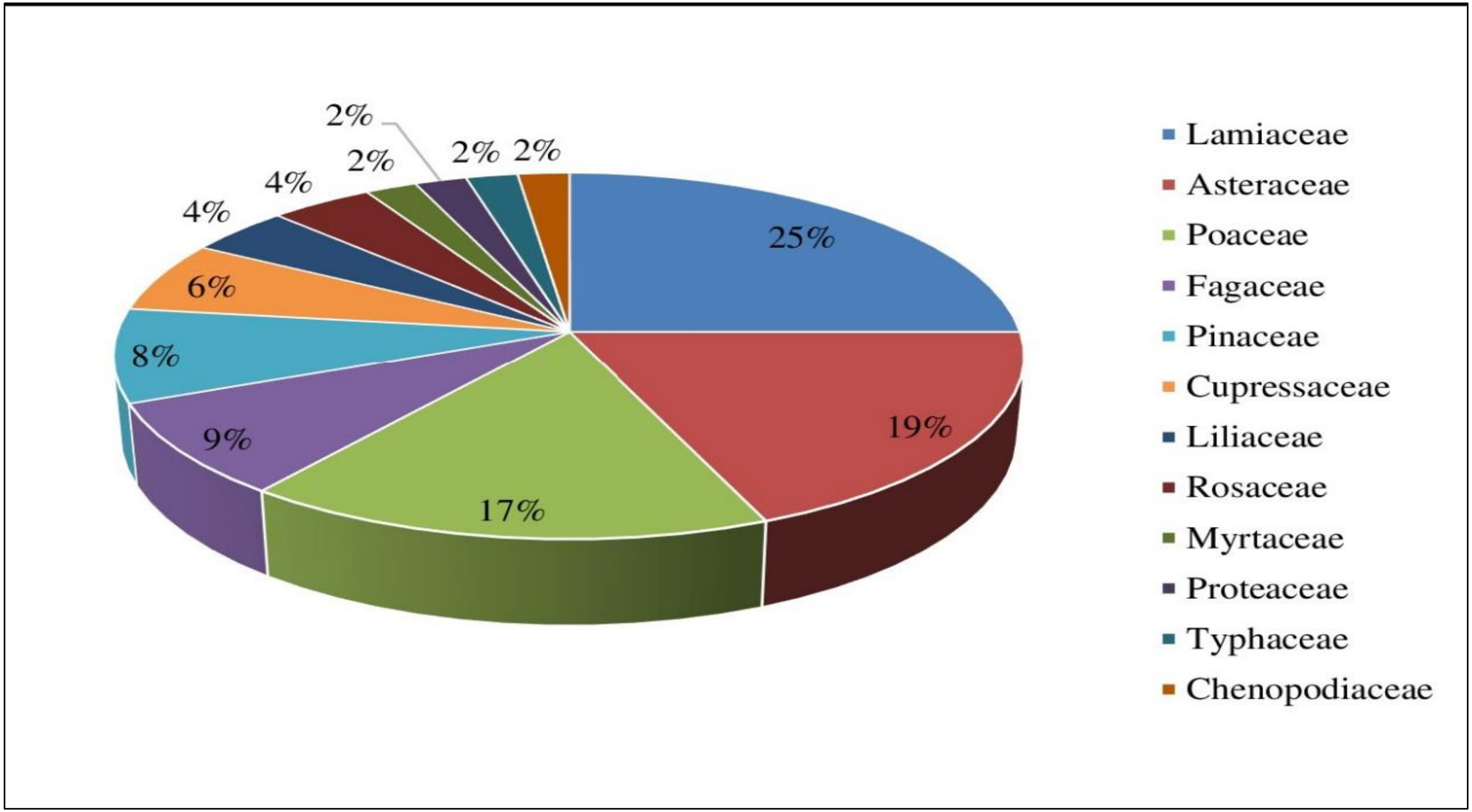

Figure 4. Families' abundance chat of the study area

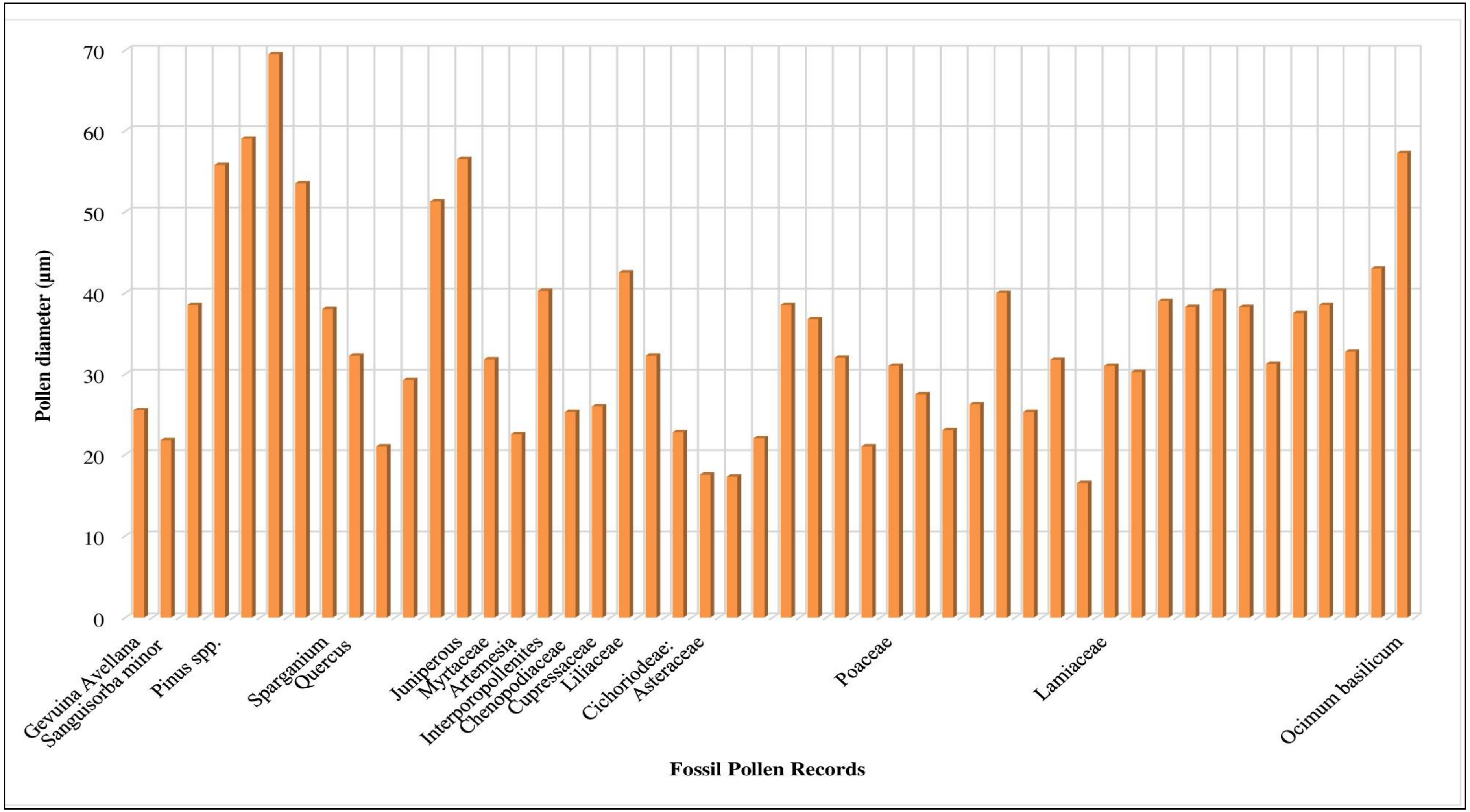

Figure 5. Pollen diameter variations in fossil pollen from late Miocene, Murree formation, Pakistan

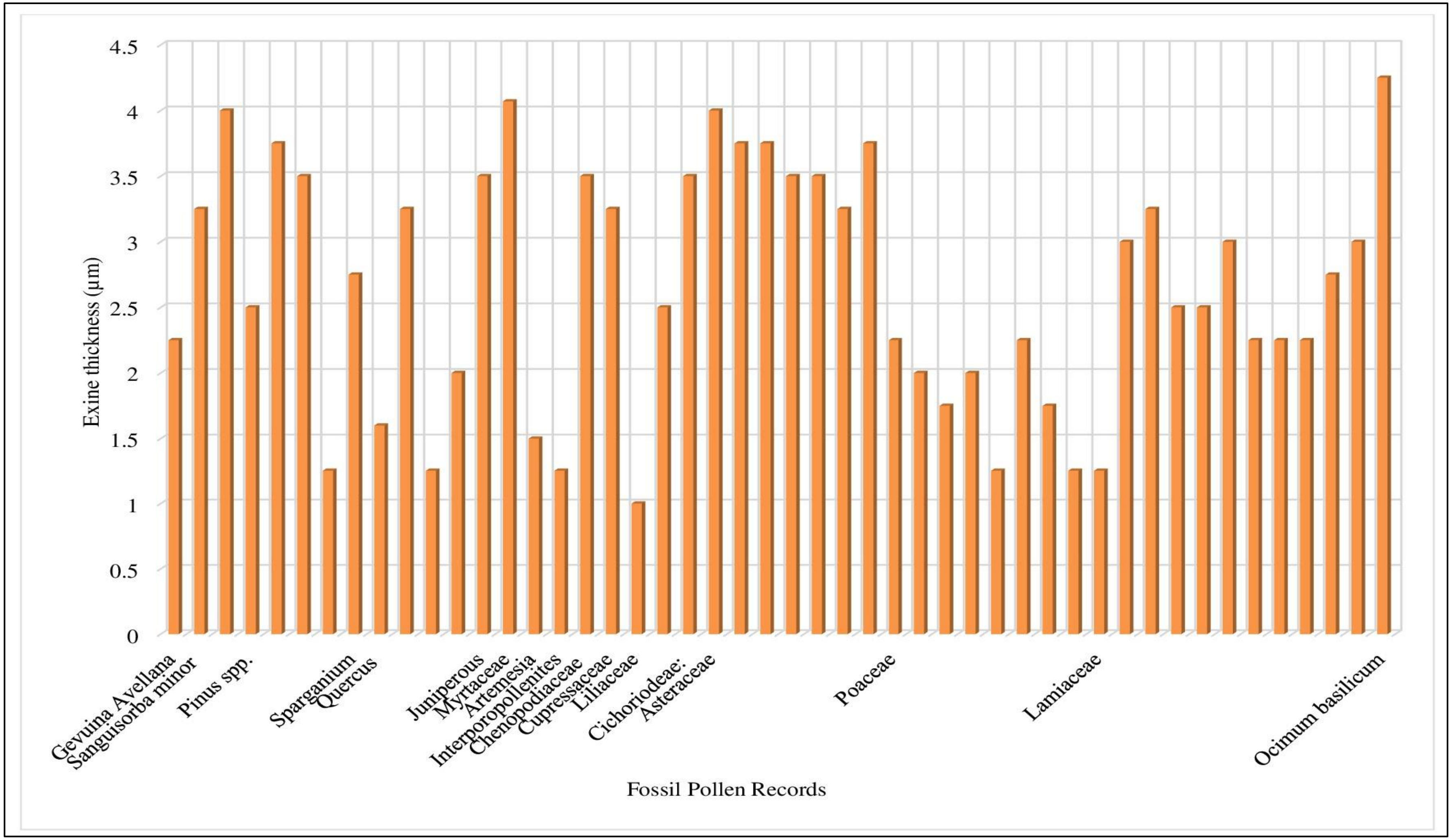

Figure 6. Variations in the values of exine thickness among fossil pollen from late Miocene, Murree formation, Pakistan

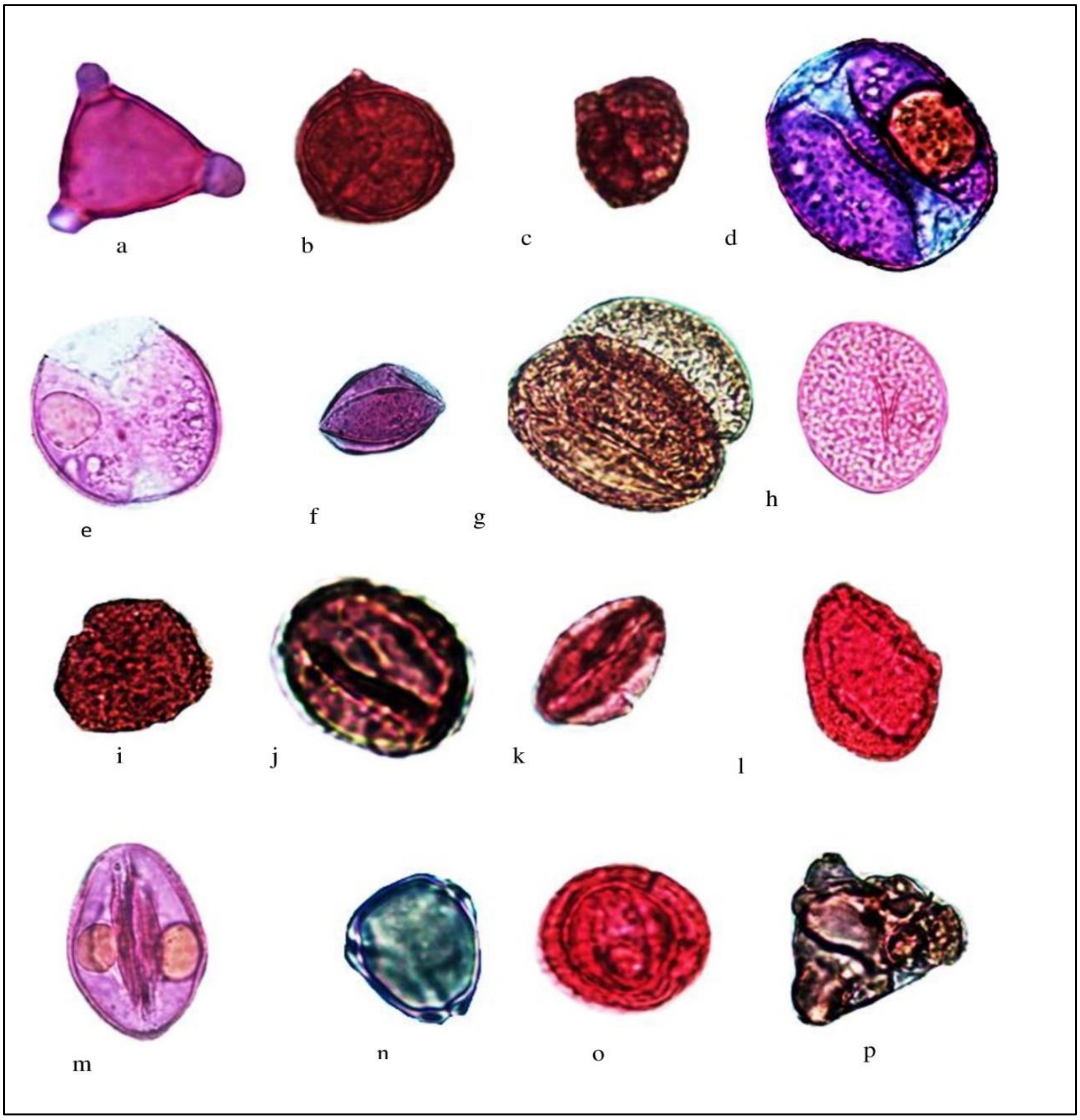

Plate 17. Light Microscopy Pollen Photographs. (a) *Gevuina Avellana* (b-c) *Sanguisorba minor* (d-g) *Pinus* sp. (h) *Sparganium* sp. (i-l) *Quercus* sp. (m) *Juniper* sp. (n) Myrtaceae (o) *Artemisia* sp. (p) *Interporopollenites* sp.

Plate 18. Light Microscopy Pollen Photographs. (a) Chenopodiaceae (b) Cupracaceae (c-d) Liliaceae (e-l) Asteraceae (m-p) Poaceae

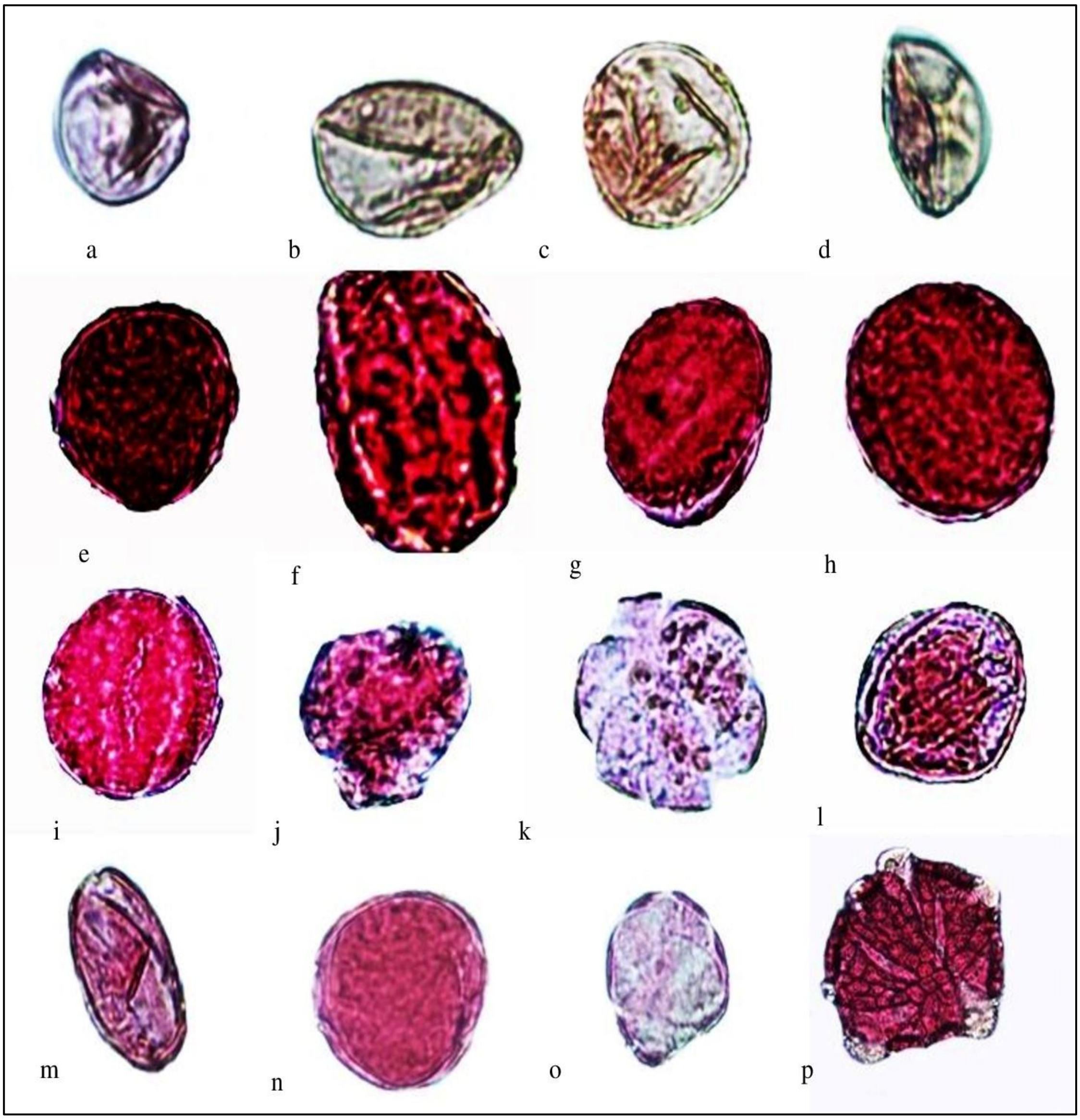

Plate 19. Light Microscopy Pollen Photographs. (a-d) Poaceae (e-p) Lamiaceae

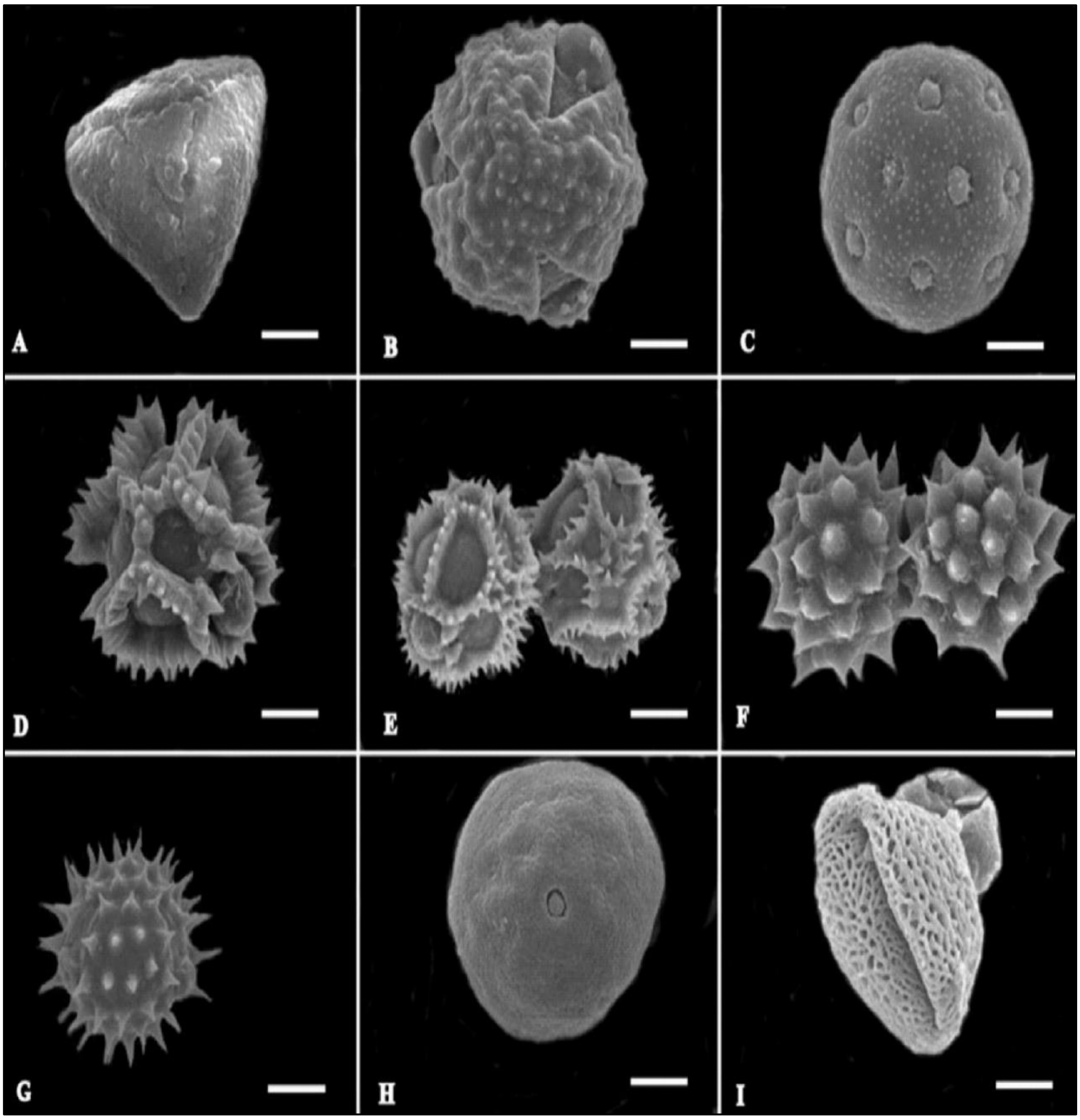

Plate 20. Scanning Electron Microscopy Pollen Photographs. (A) Myrtaceae (B) *Artemesia* spp. (C) Chenopodiaceae (D) Cichoriodeae (E-G Asteraceae (H) Poaceae (I) Lamiaceae

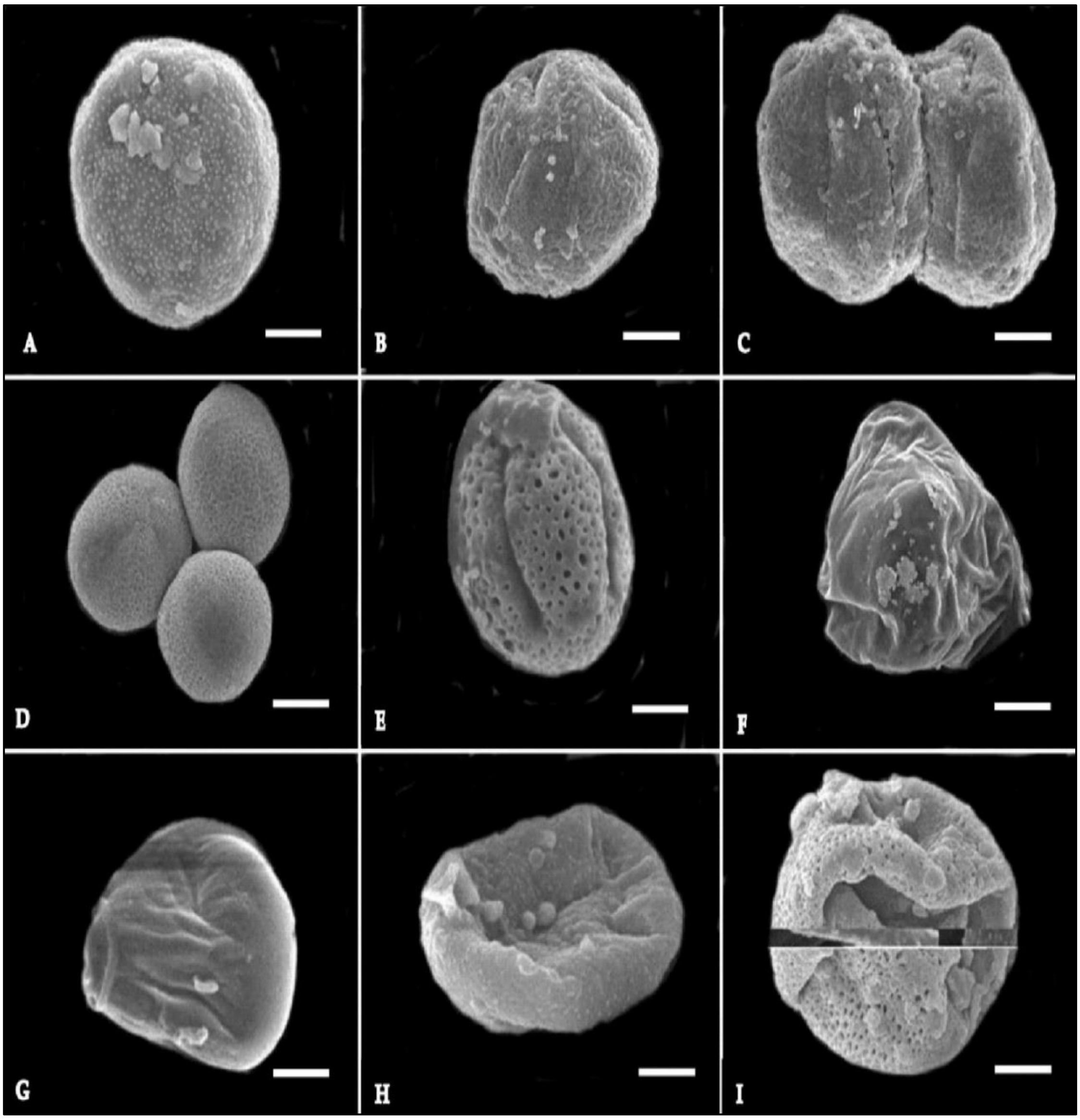

Plate 21. Scanning Electron Microscopy Pollen Photographs. (A-I) Lamiaceae

3.2 Middle Miocene Murree Formation

Using microscopic techniques, the diagnostic characters of palynomorphs were recorded from diverse families (Figure. 6). The pollen terminology was described using previously published literature (Punt, *et al*. 2007). In this study, a total of 65 sporomorphs were reported, out of which 32 were identified up to the species, genera, and then family levels. Qualitative and quantitative features of palynomorphs were shown in table 4 and 5 and their micrographs were shown in the plate 5 and 6. The morphological characters of palynomorphs were examined, which are very important to study regarding paleoclimate, palaeoecology, and biogeography.

3.2.1 Asteraceae

Artemisia pollen was circular, psilate ornamentations; pollen diameter 17.5 µm. and exine thickness 1.75 µm. Chichorioideae pollen grain had tricolpate, prolate-spheroidal and psilate-echinate ornamentations, pollen diameter 13.00–13.25 µm, exine thickness 2.25–4.75 µm, colpi length 2.5–5.5 µm, colpi width 9.00 µm, spine width 5.00 µm. and spine length 6.00 µm. While rest of the pollen was psilated, circular, the pollen diameter 16.25–31.75 µm, exine thickness 1.4–2 µm, spine length 3.25–6.75 µm, spine width 3.5–4.75 µm, pores length 5.0–6.25 µm and pores width 2.75–5.00 µm (Table 4, Plate 22).

3.2.2 Pinaceae

Pinus pollen was bissacate, tricolpate, prolate-semi-angular, and reticulate, with ornamentations having a diameter of 30.75–6125 µm and an exine thickness of 1.25–4.75 µm (Table 4, Plate 22, 23).

3.2.3 Poaceae

Pollen grains of Poaceae were examined and found to be circular and psilated; pollen diameter 16–30 µm and exine thickness was 2.00 µm (Table 4, Plate 23).

3.2.4 Apiaceae

Pollen grains were identified up to the family level. Pollen prolate-peroblate reticulate ornamentations, exine thickness 2-2.5 µm and pollen diameter 17.5-20.0 µm (Table 4, Plate 23).

3.2.5 Nyssaceae

Nyssa pollen was semi-angular-circular, triporate-tricolpate, with reticulate ornamentations, exine thickness 2.25-2.75 µm, pollen diameter 28.5-35.00 µm, colpi length 2.5-11.75 µm and colpi width 3.25 µm (Table 4, Plate 22).

3.2.6 Fabaceae

Pollen grains of Fabaceae were not identified up to the species and genus level. Pollen semi-angular, tricolporate, and psilate ornamentations; exine thickness 1.75 µm, pollen diameter 22.5 µm, length of colpi13.25 µm and width of colpi 5.5 µm (Table 4, Plate 23).

3.2.7 Fagaceae

The *Quercus* pollen was prolate, triporate-tricolpate, reticulate ornamentations, exine thickness 2.75-3.25 µm and pollen diameter of 19.5-23.25 µm (Table 4, Plate 22).

3.2.8 Myrtaceae

Myrtus pollen had sub-angular, tricolporate, and psilate ornamentations: pollen diameter 17.5 µm, exine thickness 2.00 µm, colpi length 5.25 µm, colpi width 9.75 µm, pores length 5.25 and pores width 6.25 µm. While the rest of the pollen noted had lobate, tricolporate, and psilate ornamentations, pollen diameter 19.5 µm, exine thickness 1.75 µm, colpi length 13.25 µm, colpi width 3.5 µm, pores length 6.00 µm and pores width 9.00 µm (Table 4, Plate 22).

3.2.9 Rosaceae

Sanguisobra minor pollen was circular, psilate ornamentations, an exine thickness of 5.0 µm and pollen diameter of 42.0 µm (Table 4, Plate 22).

3.2.10 Juglandaceae

Carya pollen has semi-angular, tricolpate, reticulate ornamentations: exine thickness 2.5 µm, pollen diameter 31.25 µm, colpi length 2.75 µm and colpi width 6.75 µm (Table 4, Plate 23).

3.2.11 Solanaceae

Pollen grains of Solanaceae were not identified up to the species and genus level. Pollen had angular, triporate, and reticulate ornamentations, exine thickness 2.25 µm, pollen diameter 13.25 µm, pores length 4.75 µm and pores width 4.25 µm (Table 4, Plate 23).

3.2.12 Amaranthaceae

Pollen grains of the Fabaceae were not identified up to the species and genus level. Pollen circular, polyproate triporate, reticulate ornamentations, exine thickness 3.00 µm and pollen diameter 3.75 µm (Table 4, Plate 23).

3.2.13 Arecaceae

Pollen grains of Fabaceae were not identified up to the species and genus level. The pollen was peroblate, reticulate ornamentations, pollen diameter of 55.75 µm and exine thickness of 3.75 µm (Table 4, Plate 23).

3.2.14 Betulaceae

The *Corylus* pollen had semi-angular, exine ornamentations psilate, tricolporate, an exine thickness of 2.25 µm, pollen diameter 37.5 µm, pores length 6 µm and pores width 1.25 µm (Table 4, Plate 23).

3.2.15 Lamiaceae

Pollen grain spheroidal, hexacolpate, reticulate ornamentations, exine thickness 2.5 µm, pollen diameter 35.5 µm, length of colpi 6.75 µm, and width of colpi was 35.5 µm (Table 4, Plate 22).

3.2.16 Convolvulaceae

The *Convolvulus* pollen had inter semi-angular, tricolpate, reticulate ornamentations, a pollen diameter of 75.75 µm, exine thickness 4.0 µm, colpi length 34.5 µm and a colpi width 31.0 µm (Table 4, Plate 22).

3.2.17 Spore

Triplanosporoites pollen was an angular, psilate sculpture with an exine thickness 2.25 µm and pollen diameter 32.5 µm (Table 4, Plate 23).

3.2.18 Discussion

In the present study, 32 palynomorphs were examined, out of which 31 contain pollen belonging to 16 different families and 1 is a spore. The sporomorphs contained 27 angiosperms, 4 gymnosperms, and 1 pteridophytic spore. Asteraceae has been recorded as the dominant family, followed by Pinaceae, Poaceae, Apiaceae, Nyssaceae, Fagaceae, Myrtaceae, Amaranthaceae, Solanaceae, Rosaceae, Juglandaceae, Convolvulaceae, Arecaceae, Lamiaceae, and Betulaceae. Similarly, tribe Chichorioidee and genera, i.e., *Pinus*, *Artemisia*, Nyssa, and *Quercus,* have been examined. The angiosperms contain 27 pollen belonging to 15 different families: Asteraceae (*Artemisia*, Cichiorideae), Rosaceae (*Sanguisobra minor*), Fagaceae (*Quercus*), Nyssaceae (*Nyssa*), Convolvulaceae (*Convolvulus*), Myrtaceae (*Myrtus*), Juglandaceae (*Carya*), Apiaceae, Poaceae, Fabaceae, Solanaceae, Amaranthaceae, Arecaceae, and Lamiaceae. In the 31 analyzed samples, 15 were barren and 16 contained various palynomorphs. Qualitative and quantitative features of palynomorphs observed through scanning and light microscopy using palaeobotanical techniques proved very helpful for species identification in the field of plant taxonomy. Pollen grains examined through light microscopy were hexacolpate, tricolporate, tricolpate, triporate and polyp orate in nature. Numerous shapes of the pollen grains were studied, i.e., circular, prolate, peroblate, angular, inter semi-angular, subangular, lobate, spheroidal, and prolate-spheroidal.

Three different kinds of exine sculptures were studied, i.e., reticulate, psilate and echinate, which are very vital for the differentiation of closely related species. Variations were examined in quantitative and qualitative characters of exine thickness, pollen diameter, spine length, spine width, pores length, and pores width (Figures 4, 5). The highest exine thickness was seen in Cichorioideae 4.75 µm and the lowest in *Pinus*

1.5 µm. The highest pollen diameter was recorded for *Convolvulus* and the lowest for Chichorioideae. The highest colpus length was recorded for *Convolvulus at* 34.5 µm and the lowest for Chichorioideae at 2.5 µm. The maximum colpus width was noted for *Convolvulus* 31 µm and the minimum for *Nyssa* 3.25 µm. The highest pores length was recorded in Asteraceae 6.25 µm and the lowest in Solanaceae 2.5 µm. Similarly, the highest pores width was examined for Fabaceae (9.5 µm and the lowest for *Corylus* 1.5 µm. Colpi were noted just in *Convolvulus*, *Carya*, *Nyssa*, *Myrtus*, Cichorioideae, Lamiaceae, and Fabaceae (Wu *et al*., 2019).

The *Pinus pollen grains* observed in the previous study were monad, oblate, bisaccate, acci nearly circular with its narrow attachment alveolate ornamentation (LM), corpus fossulate, verrucate, rugulate, and sacci perforated in nature (SEM), while the pollen grains observed in this study were bisaccate, prolate-semi-angular, and psilate-reticulate ornamentations (Grímsson & Zetter, 2011). *Quercus* pollen grains were prolate, eutectate, sculpturing perforate, micro verrucate, micro regulate (SEM), scabrate (LM), and basic sculpturing units are rod-shaped (Bouchal *et al*., 2016). The *Carya* pollen were observed in the previous study was seen to be oblate, eutectate, and triporate, with aperture alignment found on the equator and pores that were sunken, sculpturing perforate and nanoechinate (SEM), and scabrate (LM), while in our study it was noted to be semi-angular, triporate, and psilate (Jiang & Ding, 2008). Apiaceae pollen was noted as prolate, fossulate, microregulate (SEM), and scabrate (LM), while in this study prolate and psilate exine ornamentations were studied. The Cichorioideae pollen grains were examined as polygonal, spheroidal, tricolporate, perforate, echinate (SEM), and echinate sculpturing lophate (LM), while in this study we observed circular and echinated pollen grains (Zavada & de Villiers, 2000). The Fabaceae pollen was monad, circular to irregular, eutectate, tricolporate, sculpturing scarbate (LM), perforate-fossulate, and regulate (SEM). The Betulaceae pollen grains were seen to be oblate, eutectate, annulus present, tetra- to hexaporate (SEM), nanoechinate, and sculpturing scabrate (LM). Amaranthaceae: Pantoporate, pori diameter 0.7–1.0 µm (SEM), pori sunken, porioperculate, operculum adorned with two to six nanoechini; pollen, spheroidal, outline circular, pollen diameter 20–25 µm (LM, SEM); eutectate, exine 0.8–1.2 µm thick (LM), nexine thinner than sexine; sculpture. The morphological features observed through microscopic techniques proved very useful for the identification of species (Grímsson *et al*., 2015).

Palynoflora investigated in the present study belongs to diverse families and is shown as an indicator of the environment in the depositional phase of the Murree formation. The assemblage of some families like Arecacaceae and Amaranthaceae indicates the coastal placement of vegetation, and Nymphaeaceae indicates the freshwater environments of the study area (Kern, *et al*., 2013). The overall sporomorphs showed that vegetation existed in the coastal area or near it. The study revealed that most of the pollen belongs to angiosperms, some gymnosperms, and one pteridophytic spore. The past palynological record showed that a wetter climate and subtropical evergreen forests existed in China. It has been established that the Miocene climate of China was similar to the modern climate, and a similar case was reported from this study in Pakistan. The abundance of Fagaceae members, such as *Quercus* shows that vegetation's exists in drained soil. Among herbaceous taxa, some of the plants showed that vegetation exists in rocky or sandy soil in their depositional environment (Bouchal *et al*. 2016). Due to global climatic changes in the late middle to early Miocene *Quercus*, *Carya* decreased while conifers increased. Higher angiosperm species (*Fagus, Betula, Quercus, and Carya*) were dominant in the study area as deciduous types. Until the late Neogene, herbaceous plants were not dominant. The palynological assemblage for *Artemesia* was reported from the Late Miocene–Pliocene in the Taibei Depression, shelf basin of the East China Sea (Yunfa *et al*. 2011). *Carya* and *Nyssa* pollen were described as being triporate in nature, while in our study, *Carya* is triporate and *Nyssa* was triporate-tricolporated. The appearance of *Carya* and *Nyssa* as deciduous elements suggests that the climate was temperate during the cold season (Yu-Shu, 2001). The Arecaceae pollen grains were studied to have peroblate, monocolpate, and reticulate exine ornamentations. The fossil records of Betulaceae, Fagaceae, Juglandaceae, and Arecaceae have also been documented. The dominant families, i.e., Asteraceae, Poaceae, Pinaceae, Nyssaceae, and Fagaceae, in the study area is in accordance with the previous study carried out (Yang, Wang, Shu, & Chen, 2018). The study provides details about the vegetation variations that occurred in the Miocene epoch through the compilation of published research articles and a comprehensive literature review (Strömberg, 2011).

Three different palynological successions were established in the study area: the early Miocene-prominent period for Pinaceae; the middle Miocene-predominant angiospermic plants; and the late Miocene-period of dominant herbaceous taxa (hair

Guo, Liang, Yong, & hair Ping, 2003). Based on the mutual interactions between evolutionary trends in vegetation and climatic changes along with the geographical history of important species, i.e., *Artemisia*, these established connections can be linked with palynological assemblages in this study (Jiang & Ding, 2008). The study provides details about the vegetation variations that occurred in the Miocene epoch through the compilation of published research articles and a comprehensive literature review. The morphological features of sporomorphs were identified as preserved with fair status in sedimentary rocks. The study revealed that angiosperm taxa were comparatively dominant over gymnosperms and other lower vascular plants in the Miocene strata of the Murree Formation. It has been concluded that the present palynological studies have some similarities with the previously published literature in different areas of the world (Guo *et al*., 2003).

Table 4. Quantitative features of fossil plants pollen from middle Miocene, Murree Formation, Pakistan.

S. No	Species Name	Exine thickness (µm)	Pollen diameter (µm)	Colpi length (µm)	Colpi width (µm)	Spine’s length (µm)	Spine’s width (µm)	Pores length (µm)	Pores width (µm)
1.	*Sanguisobra minor*	4	42	-	-	-	-	-	-
2.	*Artemisia*	1.75	17.5	-	-	-	-	-	-
3.	*Convolvulus*	4	75.75	34.5	31	-	-	-	-
4.	*Carya*	2.5	31.25	2.75	6.75	-	-	-	-
5.	*Nyssa*	2.75	28.5	11.75	3.25			3.75	5.75
6.		2.25	35		-	-	-	-	-
7.	*Quercus*	2.75	23.25		-	-	-	-	-
8.		3.25	19.5		-	-	-	-	-
9.	Lamiaceae	2.5	35.5	6.75	7.25	-	-	-	-
10	*Myrtus*	2	17.5	5.25	9.75	-	-	3.25	6.25
11	Myrtaceae	1.75	19.5			-	-	6	9
12	Chichorioideae	4.75	13.25	5.5	9	4.25	5	-	-
13		2.25	13	2.5	-	-	-	-	-
14	*Pinus*	4.75	51	-	-	-	-	-	-
15		1.5	61.25	-	-	-	-	-	-
16		2.25	30.75	-	-	-	-	-	-
17		3.25	57.5	-	-	-	-	-	-
18	Fabaceae	1.75	22.75	13.25	5.5	-	-	5.5	9.5
19	*Corylus*	2.25	37.5	-	-	-	-	6	1.5
20	Arecaceae	3.75	55.75	-	-	-	-	-	-
21	Solanaceae	2.25	13.25	-	-	-	-	2.5	2.75
22	Amaranthaceae	3	13.75	-	-	-	-	4.75	4.25
23		3.25	18	-	-	-	-	5	4.5
24	Apiaceae	2	20	-	-	-	-	-	-
25		2.5	17.5	-	-	-	-	-	-
26	Poaceae	2	30	-	-	-	-	-	-

27		2	16	-	-	-	-	-	-
28	Asteraceae	4.5	17.5	-	-	-	-	-	-
29		3.5	16.25	-	-	3.75	4.75	5.25	2.75
30		5	31.75	-	-	3.25	3.5	5	4.25
31		3.75	19.5	-	-	6.75	4.5	6.25	5
32	*Triplanosporoites*	2.25	32.5	-	-	-	-	-	-

Table 5. Micromorphological characteristics of pollen qualitative from middle Miocene, Murree formation, Pakistan

S. No	Species/Taxon/Family	Pollen shape	Pollen type	Colpi/Pore	Ornamentations	Spines
1.	*Sanguisobra minor*	Circular	-	P	Psilate	A
2.	*Artemisia*	Prolate	-	A	Psilate	A
3.	*Convolvulus*	Inter semi-angular	Tripcolpate	P	Reticulate	A
4.	*Carya*	Semi-angular	Triporate	P	Psilate	A
5.	*Nyssa*	Circular	Tricolporate	P	Psilate	A
6.		Semi-angular	Triporate	P	Psilate	A
7.	*Quercus*	Prolate	-	A	Reticulate	A
8.		Prolate	-	A	Reticulate	A
9.	Lamiaceae	Spheroidal	Hexacolpate	P	Reticulate	A
10.	*Myrtus*	Sub-angular	Tricolporate	P	Psilate	A
11.	Myrtaceae	Lobate	Tricolporate	P	Psilate	A
12.	Chichorioideae	Spheroidal	Tricolpate	P	Echinate	P
13.		Prolate-spheroidal	-	A	Psilate	A
14.	*Pinus*	Angular	-	A	Reticulate	A
15.		Angular	-	A	Reticulate	A
16.		Angular	-	A	Reticulate	A
17.		Prolate	-	A	Reticulate	A
18.	Fabaceae	Semi-angular	Tricolporate	P	Psilate	A
19.	*Corylus*	Semi-angular	Triporate	P	Psilate	A
20.	Arecaceae	Peroblate		A	Reticulate	A
21.	Solanaceae	Angular	Triporate	P	Psilate	A
22.	Amaranthaceae	Circular	Polyporate	P	Reticulate	A
23.		Circular	Polyporate	P	Reticulate	A
24.	Apiaceae	Peroblate	-	A	Psilate	A
25.		Prolate	-	A	Psilate	A
26.	Poaceae	Circular	-	P	Psilate	A
27.		Circular	-	A	Psilate	A
28.		Circular	-	P	Echinate	P

29.	Asteraceae	Circular	-	P	Echinate	P
30.		Circular	-	P	Echinate	P
31.		Circular	-	P	Echinate	P
32.	*Triplanosporoites*	Angular	-	A	Psilate	A

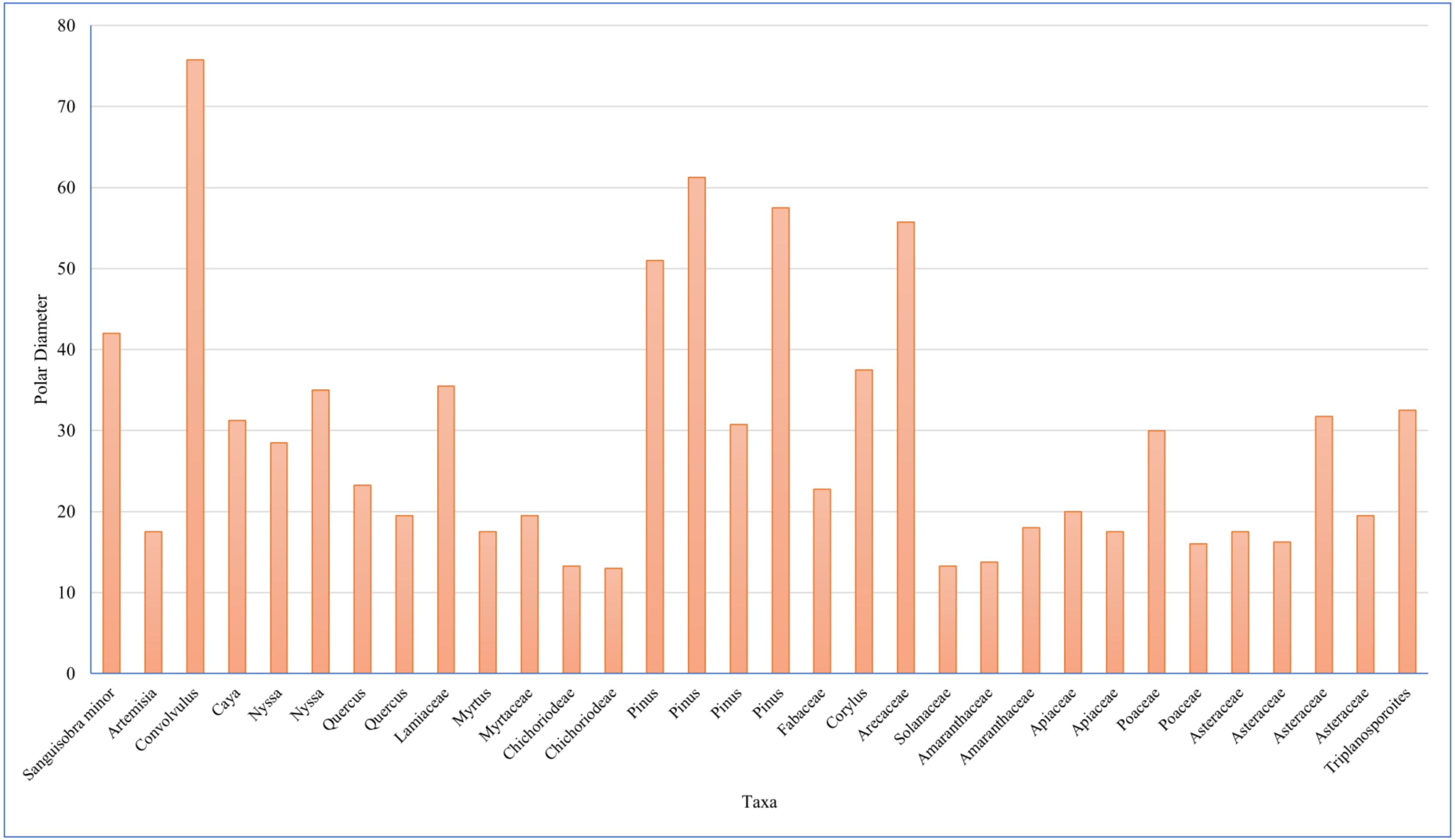

Figure 7. Pollen diameter variations in fossil plants pollen from middle Miocene, Murree formation Pakistan

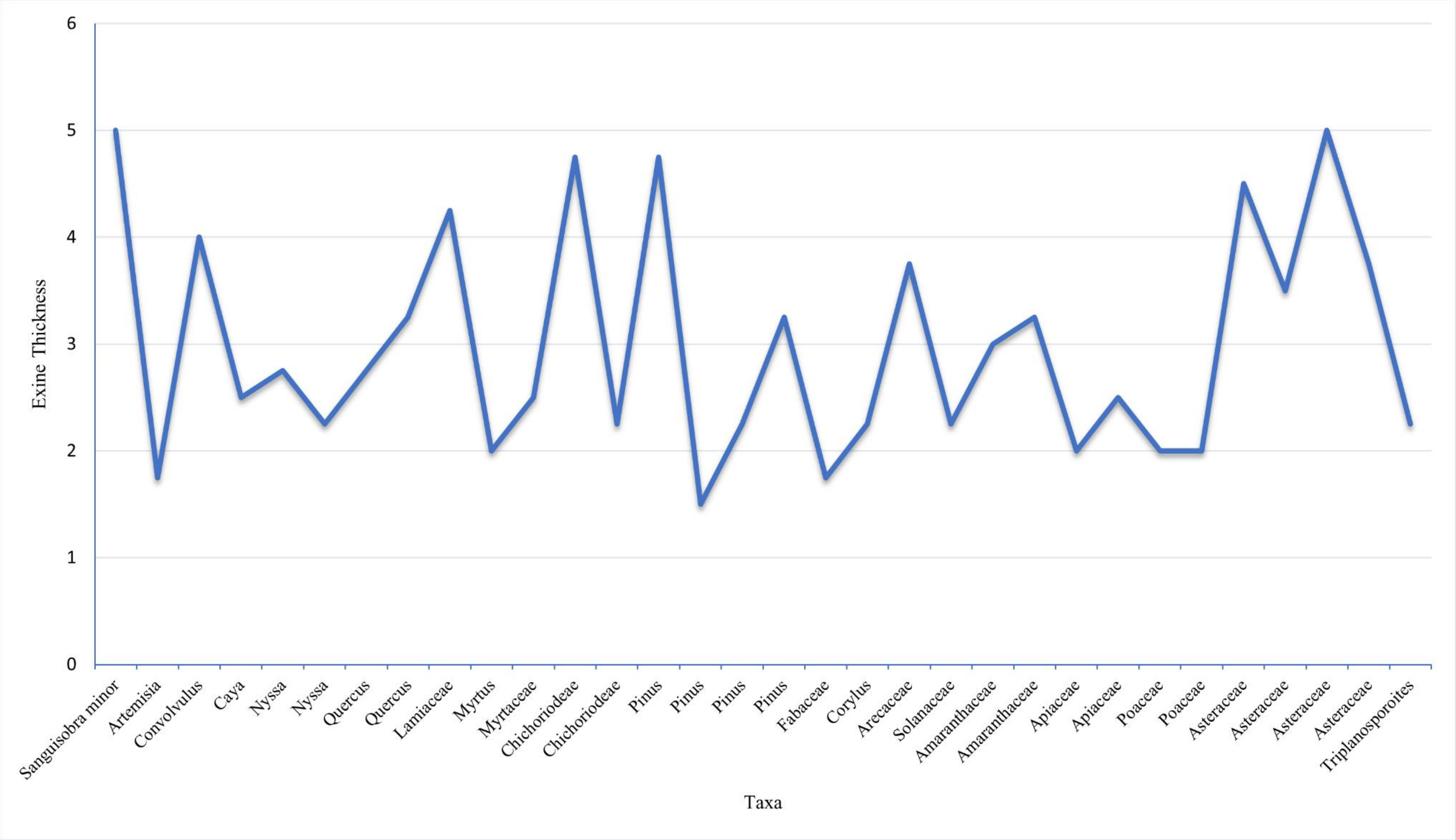

Figure 8. Variations in the values of exine thickness among fossil pollen

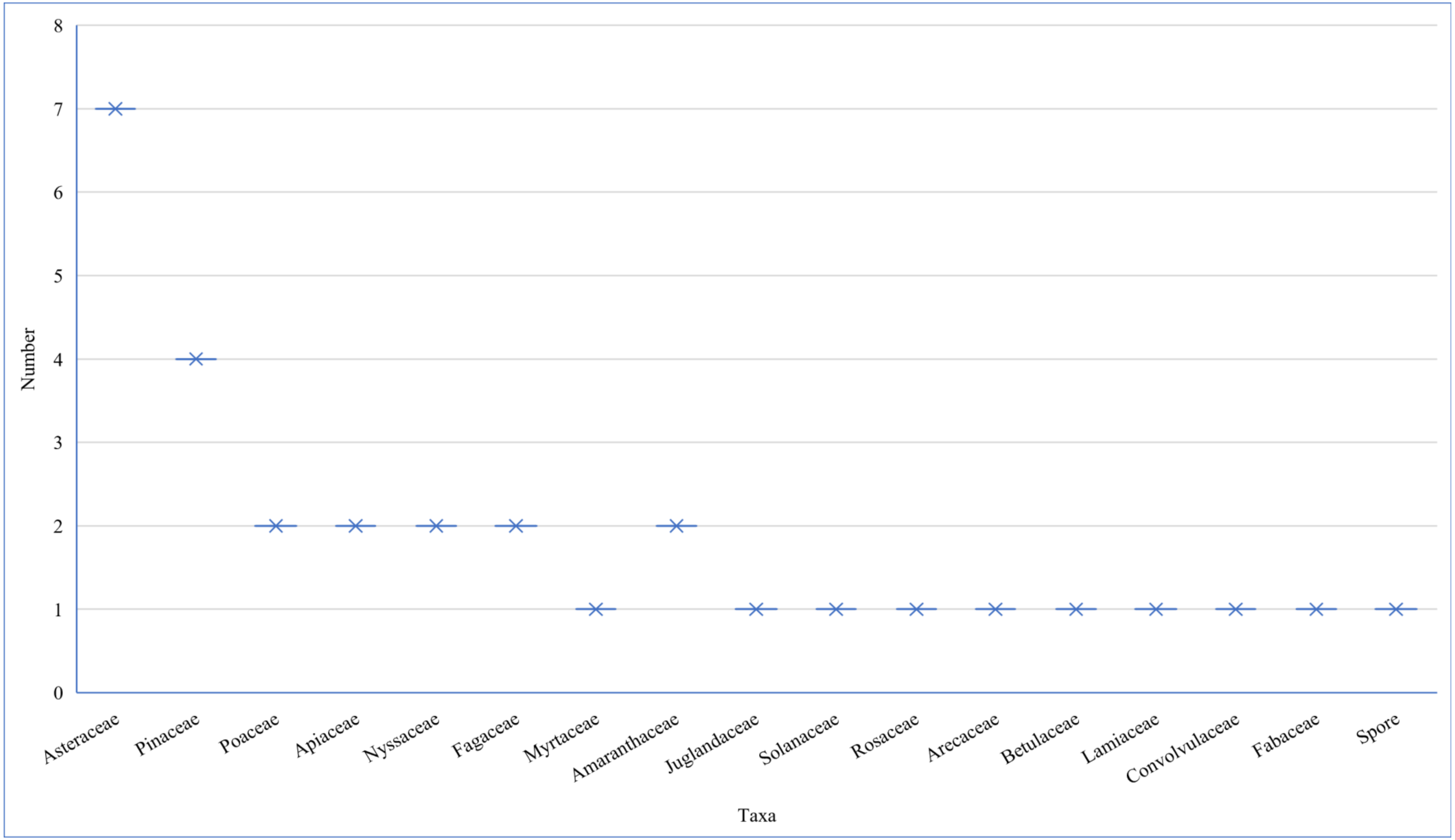

Figure 9. Family's abundance graph of the pollen grains from middle Miocene, Murree formation Pakistan

Plate 22. Light Microscopy Pollen Photographs. (a) *Sanguisobra minor* (b) *Artemisia* (c) *Convolvulus* (d) *Carya* (e-f) *Nyssa* (g-h) *Quercus* (i) Lamiaceae (j) *Myrtus* (k) Myrtaceae (l-m) Chichorioideae (n-p) *Pinus*

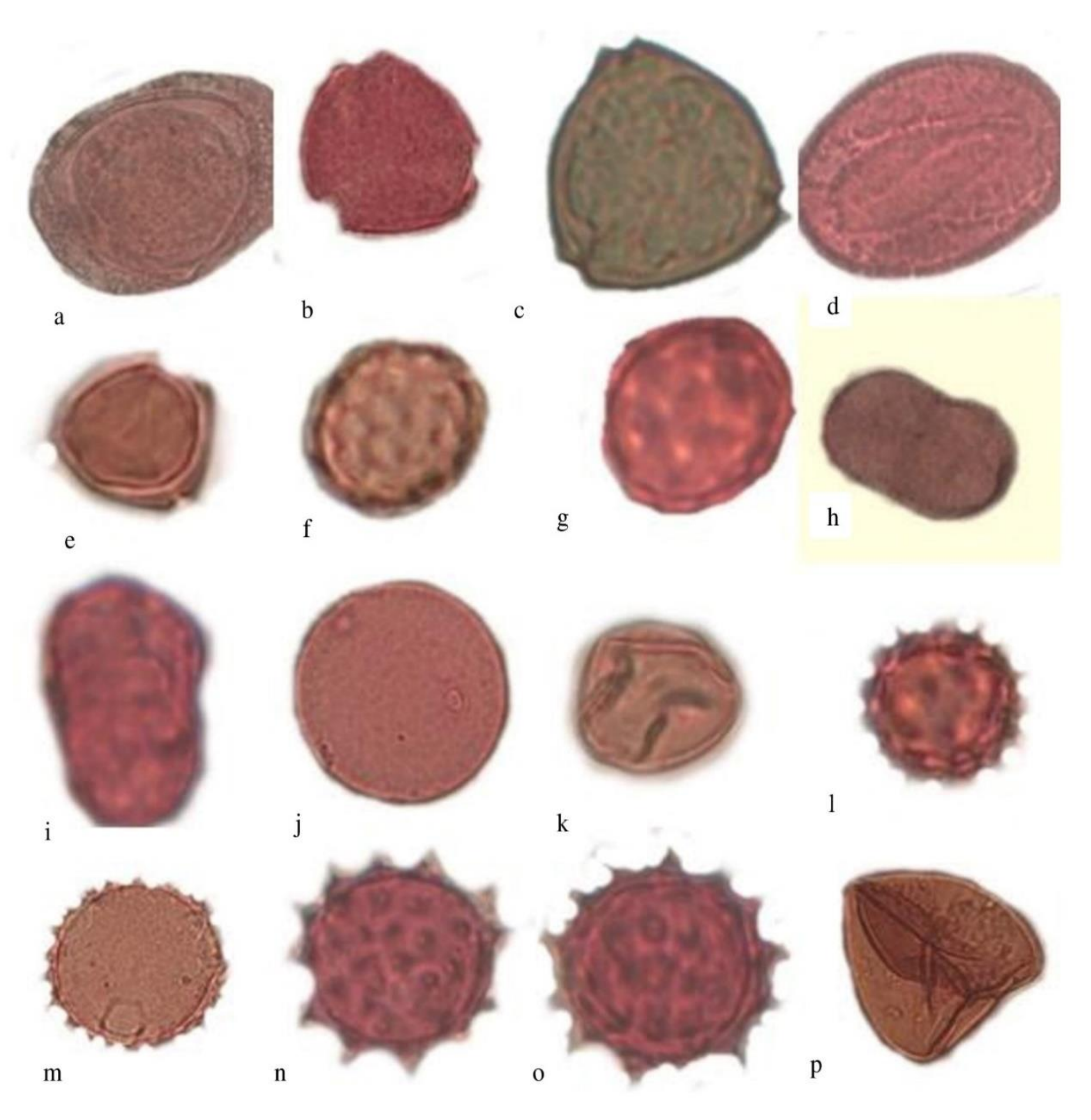

Plate 23. Light microscopy Pollen Photographs. (a) *Pinus* (b) Fabaceae (c) *Corylus* (d) Arecaceae (e) Solanaceae (f-g) Amaranthaceae (h-i) Apiaceae (j-k) Poaceae (l-o) Asteraceae (p) *Triplanosporoite*

SECTION: II

Eocene, Insect Limestone

3.1. Angiosperms basal and monocots

3.1.1. Chloranthaceae

Pollen, monad, sulcate, outline elliptic in equatorial view, heteropolar, equatorial diameter 27–30 µm (LM), 22–25 µm (SEM), exine 1.4–1.7 µm thick, nexine thinner than sexine, sculpture reticulate (LM), microreticulate to reticulate (SEM) as shown in (Table 6, Plate 24).

Remarks: Cholranthaceae pollen were widely distributed in the Cenozoic era of the Indian subcontinent, Central Africa, Europe, South America, and North America. Its pollen was examined to be inaperturate, radially symmetrical and heteropolar (Martinez *et al*., 2013).

3.1.2. Typhaceae

Type 1

Pollen, monad, outline circular in equatorial and polar view, polar axis 38–45 µm (LM), 36–39 µm (SEM), exine thickness 1.6–2.1 µm, columellae height 0.7–1.9 µm (SEM), porate, exine ornamentations reticulate (LM), microreticulate-reticulate (SEM), nanoechinate suprasculpture (SEM) as shown in (Table 6, Plate 24).

Remarks: Typhaceae pollen is an anemophilous and exists in wetland and tropical regions. Stuchlik *et al*., 2002, recorded that sporaginaceae pollenites are used for extant fossil pollen.

3.2. Angiosperms Dicots

3.2.1. Altingiaceae (*Liquidambar*)

Pollen, monad, outline circular, equatorial diameter 40–42 µm (LM), 22–32 µm (SEM), exine 2.1–2.4 µm (LM), 7.4–8.6 µm, pori length 6.4–10 µm, pori width 4.9–5.3 µm (LM), 2.5–3.2 µm (SEM), pantaporate, exine ornamentations reticulate, perforate (LM), reticulate-perforate (SEM), nexine thinner to sexine, pori elliptical shape, sculpture membrane aerolate-verrucate (SEM) as shown in (Table 6, Plate 24).

Remarks: *Liquidambar* has perforated and pantaporate pollen, as is common in Altingiaceae. The apertures of the pollen vary from elliptical to circular. Mnay leaf fragments and fossil reproductive structures have been reported from the Gleisdorf and Gratkorn regions. Jones *et al*., (1995) determined that the pori of Liquidambar are circular. The exant taxa are mostly entomophilous and grow in areas with tropical and temperate climates.

3.2.2. Aquifoliaceae (llex)

Pollen, monad, outline elliptic in equatorial view, trilobate in polar view, polar axis 24–26 µm (LM), 19–20 µm (SEM), equatorial diameter 27–28 µm (LM), 26–28 µm (SEM), exine thickness 2.7–3.4 µm (LM), isopolar, tricolpate, colpi long: sculpture baculate (LM) clavae with microstriate suprasculpture, aperture membrane clavate, sexine and nexine indiscernible (SEM) as shown in (Table 6, Plate 25).

Remarks: Pollen grains of the Aquifoliaceae have clavae or pilae exine sculpture. Palynological records of the Paleocene epoch were recorded from the Paleocene in South America, the Senonian in Asia, and the Touronian in Australia (Zhi-Chen, 2004).

3.2.3. Buxaceae (*Buxus)*

Pollen, monad, outline circular, equatorial diameter 24-27 µm (LM), 19-23 µm (SEM), exine 1.7-2.4 µm, P/E ratio isodiametric, shape spherical; exine ornamentation reticulate (LM), microreticulate (SEM) as shown in (Table 6, Plate 25).

Remarks: The current reported species quite resembles the fossil pollen of *Buxus* (Grímsson *et al*., 2015; Punt and Marks, 1991). The fossil species of *Buxu*s, having six different monotyphes, was recorded from Eurasia (Brückner (1993). The pollen of the *Buxus* is both anemophilous and entomophilous in nature and exists in temperate and tropical areas.

3.2.4. Caprifoliaceae (Viburnum)

Pollen, monad, outline elliptical in equatorial view, lobate in polar view, polar axis 18-20 µm (LM), 16–18 µm (SEM), equatorial diameter 21–23 µm (LM), 15–16 µm (SEM), exine 2.4–2.7 µm (LM), columellae height 1–1.2 µm (SEM), P/E ratio prolate, tricolpate, exine ornamentation reticulate (LM), reticulate-microreticulate (SEM) as shown in (Table 6, Plate 25).

Remarks: Its species show similarities to the previous record of *Viburnum*. However, *V. Clemensiae* a tropical taxon native to Borneo, is the best match when considering size, muri, and columellae (Hofmann, 2018).

3.2.5. Nyssaceae

Type I

Pollen, monad, outline circular in equatorial view, triangular in polar view, equatorial diameter 25–28 µm (LM), 20–22 µm (SEM), polar axis 25–27 µm (LM), 22–24 µm (SEM), exine 2.2–2.4 µm thick, P/E ratio isodiametric, tricolpate, scarbate-reticulate (LM), reticulate, perforate-fossulate (SEM) as shown in (Table 6, Plate 26).

Type II

Pollen, monad, outline elliptical in equatorial view, equatorial diameter 20–31 µm (LM), 20–21 µm (SEM), polar axis 35–38 µm (LM), 31–32 µm (SEM), exine 3.0–4.8 µm thick, P/E ratio prolate, nexine and sexine thickness are equal, sculpture scarbate (LM), perforate-fossulate (SEM) (Table 6, Plate 26).

Remarks: The reported pollen grains show similarities to that of living Nyssaceae members (Saito *et al*., 1992). Stuchlik *et al*., 2014 studied the fossil record of scattered pollen of *Nyssa*. Members of the Nyssaceae are entomophilous and exist in swamps (eFloras, 2008).

3.2.6. Ericaceae

Type I

Pollen, tetrad, outline quadrangular in equatorial view; equatorial diameter 34–39 µm (LM), 24-29 µm (SEM); exine 2.2–2.9 µm thick (LM); tricolporate; sculpture irregularly scabrate (LM); P/E ratio isodiametric; granulate, fossulate, nanoechinate suprasculpture (SEM) as shown in (Table 6, Plate 26).

Type II

Pollen, tetrad, outline quadrangular in equatorial view, lobate in polar view; equatorial diameter 22–24 µm (LM), 18–20 µm (SEM); tricolporate; exine 1.4–1.9 µm thick (LM); sculpture irregularly scabrate (LM), P/E ratio isodiametric, areolate-verrucate, granulate, nanoechinate suprasculpture (SEM) as shown in (Table 6, Plate 26).

Type III

Pollen, tetrahedral tetrad, outline quadrangular in equatorial view, equatorial diameter 32–36 µm (LM), 19–22 µm (SEM); exine 1.7–2.7 µm thick (LM); tricolporate; ornamentation scabrate (LM), areolate-fossulate (SEM) as shown in (Table 6, Plate 27).

Remarks: The reported pollen grains resemble the fossil pollen of *Erica bergiana* due to its tetrahedral shape and exine sculpture (Halbritter and Buchner, 2016b). Its species were entomophilous in nature and grow in alpine meadows, swamps, and bogs.

3.2.7. Eucommiaceae (Eucommia)

Pollen, outline elliptical in equatorial view, trilobate in polar view (LM), polar axis 29–32 µm (LM), 20–23 µm (SEM), equatorial diameter 33–36 µm (LM), 22–24 µm (SEM), exine 2.7–3.6 µm thick (LM), tricolporate, P/E ratio prolate, colpi elongated, sculpture psilate, psilate-regulate (SEM) as shown in (Table 6, Plate 27).

Remarks: Paleopalynological record of *Eucommia from the* Eocene of America, Mexico, and Canada has been recorded. Auto fluorescent elastic latex filaments are well preserved with many remaining and resemble the *Eucommia* with many characters (Call *et al.*, 1997).

3.2.8. Euphorbiaceae

Pollen, monad, outline elliptical in equatorial view, lobate in polar view, polar axis 36.2–39.7 µm (LM), 25–27 µm (SEM), equatorial diameter 39–41 µm (LM), 34–37 µm (SEM), exine 2.7–3.7 µm long, P/E ratio prolate, tricolporate, ornamentation reticulate-perforate (LM), perforate-microreticulate (SEM) as shown in (Table 6, Plate 28).

Remarks:

Euphorbiaceae species have significant variations in pollen when examined through LM and SEM. Its species were anemophilous and grow in temperate and tropical areas (Takahashi, 2000).

3.2.9. Fagaceae

Castaneoideae Type 01

Pollen, monad, outline elliptical in equatorial view, tricolporate, sexine, and nexine of equal thickness, equatorial diameter 11–13 µm (LM), 27–32 µm (SEM), exine 2.7–3.4 µm thick, polar axis 43–47 µm (SEM), 20–30 µm (LM), P/E ratio prolate, sculpture psilate (LM), psilate-regulate (SEM) as shown in (Table 6, Plate 28).

Remarks: The reported pollen resembles in tricolpate aperture and exine sculpture to the fossil pollen of Fagaceae (Denk, 2003). Its species were anemophilous and found in temperate climates and well drained forests (eFlora, 2008).

Quercus type I

Pollen, monad, outline elliptical in equatorial view, lobate in polar view, equatorial diameter 22–24 µm (LM), 16–18 µm (SEM), exine 2.2–2.4 µm thick, polar axis 26–33 µm (LM), 27–30 µm (SEM), P/E ratio prolate, tricolporate, sexine thinner to nexine, sculpture scabrate-perforate (LM), verrucate-fossulate, perforate (SEM) as shown in (Table 6, Plate 28).

Quercus type II

Pollen, monad, outline elliptical in equatorial view, lobate in polar view, equatorial diameter 20–22 μm (LM), 14–16 μm (SEM), exine 1.8–2.2 μm thick, polar axis 24-28 μm (LM), 21–24 μm (SEM), P/E ratio prolate, tricolporate, sexine thinner to nexine, sculpture scabrate-perforate (LM), verrucate-fossulate and perforate (SEM) as shown in (Table 6, Plate 29).

Remarks: The pollen grains of *Quercus* were tricolpate in nature and large. Species were distributed in the sediments of tertiary deposits. Some of the species are reported from the Taizhou Formation in China (Zhi-Chen, 2004).

3.2.10. Hamelidaceae

Pollen, monad, outline elliptical in equatorial view, trilobate, polar axis 28–31 μm (LM), 27–31 μm (SEM), equatorial diameter 29–36 μm (LM), 21–32 μm (SEM), exine 1.4–3.10 μm thick, P/E ratio prolate, tricolpate, nexine thinner than sexine, ornamentation reticulate (LM), micro-reticulate-fossulate, nanoechinate suprasculpture (SEM) as shown in (Table 6, Plate 29).

Remarks: Pollen grains of the Hamelidaceae were reported to be triolporate or tricolpate in nature. It was recorded from the Eocene-Pliocene in France and Russia (Muller, 1981).

3.2.11. Juglandaceae

Carya

Pollen, monad, outline spherical to weakly triangular in polar view, elliptical in equatorial view, polar axis 36–44 μm (LM), 28–33 μm (SEM), equatorial diameter 28–34 μm (LM), 25–27 μm (SEM), exine 1.4–2.1μm thick, ornamentation psilate (LM), granulated (SEM), porus membrane granulate (SEM) (Table 6, Plate 29).

Remarks: Palynological records of Juglandaceae from the Paleocene have been recorded from Europe and North America (Muller, 1981). In China, *Carya cathayensis, Caryapollenites simplex* were widely distributed in the upper tertiary deposits.

Type 1

Pollen, monad, outline triangular in polar view, elliptical in equatorial view, equatorial diameter 16–26 µm (LM), 12–14 µm (SEM), exine 1.2-1.7 µm thick, polar axis 24-27 µm (LM), 25–27 µm (SEM), ornamentation psilate (LM), granulate-nanorugulate (SEM), porus membrane granulate (SEM) as shown in (Table 6, Plate 30).

3.2.12. Loranthaceae

Pollen, monad, triangular in polar view, polar axis 28–31 µm (LM), 22–15 µm (SEM), equatorial diameter 25–28 µm (LM), 18–25 µm (SEM), exine 1.37–3.27 µm thick, tricolpate; sculpture psilate (LM), microbaculate, no colpus membrane (SEM) as shown in (Table 6, Plate 30).

Remarks: Paleopalynological record of Loranthaceae has been reported from the Eocene of Germany and America. The extant members of *Loranthus* were investigated in tertiary deposits and the Liushagang Formation in China (Zhi-Chen, 2004).

3.2.13. Malvaceae

Tilioideae_type_01

Pollen, monad, outline circular in polar view, elliptical in equatorial view, equatorial diameter 22–31 µm (LM), 24-29 µm (SEM), exine 1.4–2.5 µm thick, polar axis 28–30 µm (LM), 23–26 µm (SEM), P/E ratio prolate, triporate, exine ornamentation scabrate-perforate (LM), nanoreticulate-perforate (SEM) shown in (Table 6, Plate 30).

Remarks: The fossil pollen of Malvaceae had a wide ecological range and distribution in the northern hemisphere, including riparian forests. Many members of the family, i.e., *Tilia* and *Cragaria*, resemble each other in many morphological features (eFlora, 2008; Kvaček *et al*., 2002).

3.2.14. Myricaceae

Myrica

Pollen, monad, outline elliptical in equatorial view, triangular in polar view, polar axis 26–30 µm (LM), 24–25 µm (SEM), equatorial diameter 26–32 µm (LM), 23–24 µm (SEM), exine 1.9–2.9 µm thick, sexine usually thicker than nexine, pores circular and funnel shaped, sculpture psilate (LM), scarbate-miroechinate (SEM) as shown in (Table 6, Plate 31).

Remarks: Myricaceae were widely spread in the northern hemisphere. Some of the members have recorded from Luhutai and Lingfeng formations in the Paleocene (Liu, 1983)

3.2.15. Oleaceae

Type I

Pollen, monad, outline circular-elliptical in equatorial view, lobate in polar view, equatorial diameter 22–27 µm (LM), 15–18 µm (SEM), exine 2.4–3.4 µm thick, polar axis 26–28 µm (LM), 21–28 µm (SEM), columellae height 0.7–1.1 µm (SEM), P/E ratio prolate, tricolpate, sculpture reticulate (LM), reticulate-fossulate, muri nanorugulate (SEM) as shown in (Table 6, Plate 31).

Type II

Pollen, monad, outline lobate in polar view, circular-elliptical in equatorial view, tricolporate, equatorial diameter 17-20 µm (LM), 13-14 µm (SEM), exine 5-8 µm thick, polar axis 17–19 µm (LM), 11–12 µm (SEM), P/E ratio isodiametric, ornamentation reticulate (LM), microreticulate to reticulate, nanoechinate suprasculpture (SEM) as shown in (Table 6, Plate 31).

Remarks: The fossil record of the Oleaceae shows reticulate exine sculpture, an oblong body, and a circular shape. The fossil records of pollen were investigated from the Oligocene Upper Ganchaigou Formation and the Eocene Lower Ganchaigou Formation (Punt and Marks, 1991).

3.2.16. Platanaceae

***Platanus* I**

Pollen, monad, lobed in polar shape, circular-elliptical in equatorial shape, polar axis 23–24 µm (LM), 18-20 µm (SEM), equatorial diameter 22–24 µm (LM), 18–20 µm (SEM), exine 2-2.7 µm thick (LM), tricolporate, exine ornamentation scabrate-reticulate (LM), microreticulate-reticulate (SEM) as shown in (Table 6, Plate 32).

***Platanus* II**

Pollen, monad, lobed in polar shape, circular-elliptical in equatorial shape, polar axis 25–27 µm (LM), 20-22 µm (SEM), equatorial diameter 23-24 µm (LM), 19-21 µm (SEM), exine thickness 2.3-2.9 µm, P/E ratio oblate, tricolporate, exine ornamentation scabrate-reticulate (LM), microreticulate-reticulate (SEM) as shown in (Table 6, Plate 32).

Remarks:

The fossil members of *Platanus* resemble to the extant in many morphological features, including exine sculpture, colpus, and aperture margin (Denk and Tekleya, 2006). These were anemophilous in nature and existed in Southeast Asia, Eurasia, and America (eFlora, 2008).

3.2.17. Rosaceae

Pollen, monad, P/E ratio prolate, outline elliptical in equatorial view, tricolporate, polar axis 17–18 µm (LM), 19–20 µm (SEM), equatorial diameter 18–20 µm (LM), 16–17 µm (SEM), exine 1.8–3.4 µm long, P/E ratio prolate, sculpture psilate (LM), striate (SEM) as shown in (Table 6, Plate 32).

Remarks: Morphological features of Rosaceae pollen have been investigated from the Eocene-Oligocene Ganehaigou Formation in China and South Africa (Zhi-Chen, 2004).

3.2.18. Rutaceae

Type 0I

Pollen, monad, lobate in polar view, elliptical in equatorial view, equatorial diameter 22–28 µm (LM), 14–17 µm (SEM), exine 1.9–2.7 µm thick, polar axis 19–23 µm (LM), 20–23 µm (SEM), tricolpate, sexine is thick like nexine, semitectate; reticulate (LM), reticulate-striatoreticulate-striatoreticulate (SEM) shown in (Table 6, Plate 33).

Remarks: The palynological investigations of current reported Rutaceae pollen corresponds to the extant pollen (Cao *et al*., 2014).

3.2.19.

Salicaceae

Salix

Pollen, monad, outline lobed in polar view, elliptical in equatorial view, equatorial diameter 21–24 µm (LM), 11–12 µm (SEM), exine thickness 1.7–2.9 µm, polar axis 23–26 µm (LM), 21–23 µm (SEM), columellae height 0.4–0.8 µm (SEM), tricolpate; exine ornamentation reticulate (LM), aerolate-reticulate (SEM) shown in (Table 6, Plate 33).

Remarks: The fossil pollen of *Salix* showed crested reticulate exine ornamentation. Stuchlik *et al*., 2009) used *Salixipollenites* for some of the *Salix* pollen grains.

3.2.20. Santalaceae

Type I

Pollen, monad, outline trilobite in polar view, elliptical in equatorial view, equatorial diameter 20–22 µm (LM), 16–17 µm (SEM), exine 1.2–2.4 µm thick, polar axis 20–21 µm (LM), 17–18 µm (SEM), exine ornamentations psilate, echinate (LM & SEM) as shown in (Table 6, Plate 33).

Type II

Pollen, monad, outline lobate in polar view, circular in equatorial view, equatorial diameter 26–27 µm (LM), 19–20 µm (SEM), exine 1.9–2.7 µm thick, polar axis 25–28 µm

(LM), 23–24 μm (SEM), P/E ratio isodiametric, exine ornamentation psilate, echinate (LM & SEM) as shown in (Table 6, Plate 34).

Remarks: Palynological record of the Sanatales has been investigated by Hawksworth and Wiens (1972) using LM and SEM and also the phylogenetic linkage of *Arceuthobium* has been reported.

3.2.21. Styracaceae

Type I

Pollen, monad, outline triangular in polar view, equatorial diameter 21–27 μm (LM), 11–16 μm (SEM), polar axis 22–37 μm (LM), 23–27 μm (SEM), exine 2.7–4.8 μm thick, P/E ratio prolate, tricolporate, exine ornamentation psilate-scabrate (LM), perforate-aerolate (SEM) as shown in (Table 6, Plate 34).

Type II

Pollen, monad, outline trilobite in polar view, elliptical in equatorial view, polar axis 21–25 μm (LM), 13–16 μm (SEM), exine 1.7–2.7 μm thick, equatorial diameter 26–33 μm (LM), 13–17 μm (SEM), P/E ratio prolate, tricolpate, exine ornamentation scabrate (LM), microreticulate, perforate-aerolate (SEM) as shown in (Table 6, Plate 34).

Type III

Pollen, monad, outline prolate in equatorial view, angular-oblate in polar view, equatorial diameter 21–24 μm (LM), 16–17 μm (SEM), polar axis 21–28 μm (LM), 20–21 μm (SEM), exine 3.1–4.8 μm thick, P/ E ratio prolate, tricolpate, psilate-perforate (LM), regulate, perforate-aerolate (SEM) as shown in (Table 6, Plate 35).

Type IV

Pollen, monad, outline triangular in polar view, elliptical in equatorial view, equatorial diameter 26–29 μm (LM), 22–23 μm (SEM), polar axis 27–29 μm (LM) 27–28 µm (SEM), exine 3–4 µm thick, P/ E ratio prolate, exine ornamentation psilate, perforate (LM), perforate-aerolate (SEM) as shown in (Table 6, Plate 35).

Type V

Pollen, monad, outline elliptical in equatorial view, triangular in polar view, equatorial diameter 23–24 µm (LM), 17–18 µm (SEM), polar axis 26–29 µm (LM), 20–21 µm (SEM), exine 3.4–4.3 µm thick, tricolporate, exine ornamentation psilate (LM), perforate-aerolate (SEM) as shown in (Table 6, Plate 35).

Remarks: The extant record of *Rhododendron* pollen has been investigated (Morton and Dickison, 1992). These were entomophilous and existed in deciduous forests (Hwang and Grimes 1996).

3.2.22. Symplocaceae

*Symplocos*_type_01

Pollen, monad, outline elliptical in equatorial view, equatorial diameter 29–38 µm (LM), 15–16 µm (SEM), polar axis 30-38 µm (LM), 21–22 µm (SEM), exine 1.7–2.6 µm thick, tricolpate, P/E ratio prolate, exine ornamentation scarbate (LM), gemmate-verrucate and fossulate (SEM) as shown in (Table 6, Plate 36).

Remarks: The reported pollen grains resembles to the fossil and extant pollen of *Sympolocos* in exine ornamentations and aperture configuration. Its specie swere mostly entomophilous and lived in waterlogged soils (eFlora, 2008).

3.2.23. Thymelaeaceae

Type_01

Pollen, monad, outline circular in polar view, equatorial diameter 25–26 µm (LM), 17–18 µm (SEM), polar axis 23–26 µm (LM), 16–17 µm (SEM), exine 4.2-5.8 µm long, exine ornamentation psilate-echinate (LM & SEM) as shown in (Table 6, Plate 36).

Remarks: Palynological records of fossil pollen were investigated in the Cenozoic Liushagang Formation, and the extant species showed minimum exine thickness as compared to the recent (Zhi-Chen., 2004).

3.2.24. Ulmaceae

Cedrelospermum

Pollen, monad, outline elliptical to irregular in polar view, polar axis 23–24 μm (LM), 15–16 μm (SEM), exine 1.3–2.1 μm thick, equatorial diameter 23–26 μm (LM), 18–19 μm (SEM), P/E ratio prolate, tetra to pantaporate, exine ornamentation psilate (LM), verrucate-granulate (SEM), granulate suprastructure (SEM) as shown in (Table 6, Plate 36).

Remarks: In the Eocene, *Cedrospermum* was commonly distributed in the Neogene, and the pollen record of this family shows a broader ecological range (Manchester, 1989). Its pollen grains were seen to be porous, having verrucate exine ornamentation.

3.3. Gymnosperms

3.3. 1. Ephedraceae

Type_01

Pollen, monad, outline lobate in polar view, elliptical in equatorial view, polar axis 27–45 (LM), 35–36 μm (SEM), exine 1.4–3.3 μm thick, equatorial diameter 23–25 μm (LM), 13–14 μm (SEM), sulcate, exine ornamentation psilate (LM), scarbate, plicate (SEM) as shown in (Table 6, Plate 37).

3.3.2. Pinaceae_Cathya

Pollen, bisaccate, corpus elliptical in equatorial view, long pollen diameter: 55–59 μm (LM), 45–51 μm (SEM), short polar diameter: 22–25 μm (LM), 20–23 μm (SEM), saccus width: 15-20 μm, saccus height: 19–25 m; sexine thicker than nexine, sacci spherical, exine ornamentation of sacci and cappa: scarbate (LM), verrucate-perforate (SEM) as shown in (Table 6, Plate 37).

Rremarks: The extant species of *Cathya* are anemophilous with a broad taxonomic range (Farjon, 1990). In the Cenozoic, the genus was widely spread within the northern hemisphere (Liu & Basinger, 2000).

3.4. Spores

3.4.1. Trilete_spore_type_01

Spore, monad, outline triangular in polar view, elliptical in equatorial view, equatorial diameter 33–43 µm (LM), 26–28 µm (SEM), exine 2.7–4.1 µm thick (LM), µm (LM), aperture trilete and circular horseshoe-like, exine ornamentation psilate**,** scabrate (LM and SEM) as shown in (Table 6, Plate 37).

Remarks: It is indicated by the size range, trilete mark configuration, and microrugulate exospore sculpture (Korasidis *et al*., 2023).

3.5 Discussion

In this study, a total of 42 pollen grains belonging to angiosperm monocots, dicots, gymnosperms, and spores were identified and analyzed. Styraceae is considered to be the dominant family, having 5 species, followed by Ericaceae (03), Fagaceae (03), Nyssaceae (02), Juglandaceae (02), Plantaceae (02), Santalaceae (02) Chloranthaceae (01), Typhaceae (01), Altingiaceae (*liquidombor*-01), Buxaceae (01), Caprifoliaceae (*Viburnum*-01), Eucommiaceae (*Eucommia*-01), Euphorbiaceae (01), Hammelidaceae (01), Loranthaceae (01), Malvaceae (01), Myricaceae (01), Oleaceae (01), Rosaceae (01), Rutaceae (01), sympolaceae (01), Thymelaceae (01), Ulmaceae (01), Ephedraceae (01), Pinaceae (01) and trilete spore (01). Using the single grain method, the palynomorphs were examined with microscopic techniques. With the help of previously published literature, the palynomorphs were identified up to the genus and family level. The comparison of LM & SEM showed that the present investigated species of Styraceae resemble to the extant and fossil species of Styraceae reported from Europe (Hofmann *et al*., 2022). It is widely spread in the tropical and warm temperate areas of America, Europe, and Asia. In the current study, the pollen grains investigated were triangular, tricolporte, P/E rato prolate, and sculpture pailate-aerolate, while in the previous study they were tricolporate, prolate, and tectum regulate-fossulate. In this study, Fagaceae (*Quercus*) pollen grains were observed to be tricolporate, fosulate-regulate, and perforate, while in the previous study they were seen to be tricorporate, prolate, perforate, regulated, and microverrucate ornamentations. The pollen grains of current analyzed *Quercus* and Castaneoideae showed much similarity to the previous study. In the Eocene flora of Primorskii krai, megafossils of *Quercus, Castanea, Fagus, Lithocacarpus* and *Castanopsis* have been reported from the Eocene, showing high diversity of Fagaceae (Naryshkina & Evstigneev 2020; Hofmann, 2010). The pollen grains of the Ericacace observed in this study were quadrangular, sculpture irregularly scabrate, P/E ratio isodiametric, and granulate exine sculpture. The pollen grains studied in the previous study were tetrads collected from China, America, Germany, and Austria. Exine sculpture was regulated, and visin threads surrounded the pollen grain completely, which looked to be granular, not completely smooth, and exine sculpture was regulated (Zetter & Hesse 1996).

Pollen grains of Oleaceae observed in this study were tricorporate, sculpture reticulate, nano-echinate suprasculpture, while in previous studies it has been recorded to be tricolporate with small pores,microechinate suprasculpture, and reticulate sculpture (Kmenta & Zetter, 2013). The palynological record of *Olea, Phillyrea* and *Fraxinus* from the Cenozoic sediments of Altmittweida, Saxony and Germany have been reported (Manchester *et al*. 2015). In current research work the Loranthaceae pollen grains were seen to be tricolporated, triangular, psilated in LM, and microbaculated (SEM). Fossil pollen of Loranthaceae was observed to be triangular with concave sides, syncolpate, and having distinctive differentiation in exine sculpture and aperture configuration. The LM and SEM studies showed that it was abundant in Greenland and central Europe in the middle Eocene (Manchester *et al*. 2015). Pollen grains of Typhaceae examined in this study were porated, reticulate sculpture, and miroreticulated-reticulated sculpture while the pollen grains of Typhaceae observed in the previous study were ulcerated, reticulate sculptures with reticulum crested by nanoechini. The fossil record of the Typhaceae (*Sporangium*) was observed in China, America, New Zealand, and Australia (Punt & Clarke, 1976). Altingiaceae pollen grains examined in this study were pantaporated, reticulated, pori elliptical, while in previous studies, *Semiliquidambar, Noronha, Liquidambar* and *Altingia* were perforated, pantaporated, and nano-echinated (Ickert-Bond & 2013). Thymelaceae pollen seen in this study was monad and had psilate-echinate sculpture, while in the previous study it was examined to have a crotonoid pattern of exine, and based on its architecture, four types of pollen grains were examined using LM, SEM, and TEM (Herber, 2002). Pollen grains of Rutaceae were seen to be tricolporate, striato-reticulate, and colpus membrane granulated, which resembles the previous results of Rutaceae (Tricolporopollenites), reported from the lower Eocene of northwestern Tethyan region (Hofmann, 2011). Plantaceae pollen grains observed in this study were tricolpated, circular-elliptical, trilobate and reticulate exine sculpture, while the pollen grains examined in the previous study were trilobated, spheroidal or oblated, angular aperture, and tricolpated, colpi long, reaching polar area; lumina greater in mesocolpia, becoming smaller at colpi margins; sexine thick, reticulate, and quietly similar to the previous pollen (Korasidis *et al*., 2023).

Rosaceae pollen was seen to be tricolporated, prolate, and psilate-striate sculpture in this study, while pollen examined in the previous study was monad, tricolporate, endopori smaller than the width of colpi; colpi long, endopori circular, and thickened margins of endopori (Grímsson *et al*., 2016). Myricaceae pollen observed in present study was triangular, circular pore, funnel-shaped, and psilate-scabrate sculpture, while pollen grains observed in the previous study were seen to have triangular, triporate, and irregularly arranged micro-verrucae sculpture. Using different microscopic techniques, morphological features of *Morella* and *Myrica* were studied, which resemble our results (Punt *et al*., 1976). Pollen grains of the *Salix* in Salicaceae showed tricolpate, reticulate-aerolate columellae, while pollen grains of the fossil Salicaceae showed tricolporoidate, outline circular-elliptical in equatorial view, and exine sculpture microreticulate-verrucaed

(Hofmann, 2018). Pollen grains of the Juglandaceae were seen to be triangular in polar view, elliptical in equatorial view, and sculptured psilate-granulate, while the pollen grains of the fossil Juglandaceae were examined to be spheroidal-oblate, heteropolar, and micro-gemmae-echinate. Nowadays, the genus *Viburnum* consists primarily of deciduous temperate woody plants from the northern hemisphere and a few evergreen plants from mountainous South America and SE Asia (Winkworth and Donoghue, 2005). The pollen grain of the *llex* was distinctive, and it has been found in the Cenozoic sedimentary record all across the world (Muller, 1981). *Craigia*-type pollen (Malvaceae) in this study resembles the morphological characteristics of *Craigia hainanensis from the Changchang* Formation. It is commonly spread from the Eocene to the Pliocene within the northern hemisphere (Jin *et al*., 2009). Pollen grains of the Sympolaceae in present study were seen to be tricolporate, triangular, and having scabre-verrucate sculpture, while pollen grains seen in the prevuous study were tricolporate, triangular to trilobare, and slightly truncated apices in polar view, ambs are slightly concave in compressed form (Hofmann 2019). Some of the swamp inhabitants, such as *Myrica*, *Nyssa*-type and Salicaceae-type pollen, showed a wet environment with periodically flowing or standing water. Some of the results and botanical affiliations of our pollen taxa shown here are either novel or new for the Eocene era of Europe, and many were not previously discussed systematically.

Table 6. Quantitative features of fossil plants pollen from Insect limestone, UK.

S. No	Species Name	Equatorial diameter (µm) LM	Equatorial diameter (µm) SEM	Polar axis (µm) LM	Polar axis (µm) SEM	Exine thickness (µm) LM	Colpi length (µm) LM	Colpi width (µm) LM	Pori length (µm) LM	Pori width (µm) LM	Columellae height (µm) SEM
1.	Chloranthaceae	27–30	22–25	–	–	1.4–1.7	–	–	–	–	–
2.	Typhaceae	33–35	36–37	38–45	36–39	1.6–2.1	_	–	–	–	0.7–1.9
3.	Altingiaceae (*Liquidambar*)	40–42	22–32	–	–	2.1–2.4	–	–	6.4–10	4.9–5.3	–
4.	Aquifoliaceae (*llex*)	27–28	26–28	24-26	19–20	2.7–3.4	–	–	–	–	–
5.	Buxaceae (*Buxus*)	24-27	19-23	–	–	1.7-2.4	–	–	–	–	–
6.	Caprifoliaceae (*Viburnum)*	21-23	15-16	18-20	16-18	2.4-2.7	–	–	–	–	1-1.2
7.	Nyssaceae (Type I)	25-28	20-22	25-27	22-24	2.2–2.4	–	–	–	–	–
8.	Nyssaceae (Type II)	20–31	20–21	35–38	31–32	3.0–4.8	–	–	–	–	–
9.	Ericaceae (Type I)	34–39	24-29	–	–	2.2–2.9	–	–	–	–	–
10.	Ericaceae (Type II)	22–24	18–20	–	–	1.4–1.9	–	–	–	–	–
11.	Ericaceae (Type III)	32–36	19–22	–	–	1.7–2.7	–	–	–	–	–
12.	Eucommiaceae (*Eucommia*)	33–36	22–24	29–32	20–23	2.7–3.6	–	–	–	–	–
13.	Euphorbiaceae	39–41	34–37	36–39	25–27	2.7–3.7	–	–	–	–	–
14.	Fagaceae (Castaneoideae)	11–13	27–32	20–30	43–47	2.7–3.4	–	–	–	–	–
15.	*Quercus* Type I	22–24	16–18	26–33	27–30	2.2–2.4	–	–	–	–	–
16.	*Quercus* Type II	20–22	14–16	24-28	21–24	1.8–2.2	–	–	–	–	–
17.	Hamelidaceae	29–36	21–32	28–31	27–31	1.4–3.10	–	–	–	–	–
18.	Juglandaceae (*Carya*)	28–34	25–27	36–44	28–33	1.4–2.1	–	–	–	–	–
19.	Juglandaceae	16–26	12–14	24-27	25–27	1.2–1.7	–	–	–	–	–

20.	Loranthaceae	25–28	18–25	28–31	22–15	1.37–3.27	–	–	–	–	–
21.	Malvaceae (Tilioideae)	22–31	24-29	28–30	23–26	1.4–2.5	–	–	–	–	–
22.	Myricaceae (*Myrica*)	22–31	24-29	28–30	23–26	1.4–2.5	–	–	–	–	–
23.	Oleaceae Type I	22–27	15–18	26–28	21–28	2.4–3.4	–	–	–	–	–
24.	Oleaceae Type I	17-20	13-14	17–19	11–12	2.2–3.6	–	–	–	–	–
25.	*Platanus* I	23–24	18-20	22–24	18–20	2–2.7	–	–	–	–	–
26.	*Platanus* II	25–27	20-22	23-24	19-21	2.3–2.9	–	–	–	–	–
27.	Rosaceae	18–20	16–17	17–18	19–20	1.8–3.4	–	–	–	–	–
28.	Rutaceae	22–28	14–17	19–23	20–23	1.9–2.7	–	–	–	–	–
29.	Salicaceae (*Salix*)	21–24	11–12	23–26	21–23	1.7–2.9	–	–	–	–	0.4–0.8
30.	Santalaceae Type I	20-22	16-17	20–21	17-18	1.2–2.4	–	–	–	–	–
31.	Santalaceae Type II	26–27	19–20	25–28	23–24	1.9–2.7	–	–	–	–	–
32.	Styraceae Type I	21–27	11–16	22–37	23–27	2.7–4.8	–	–	–	–	–
33.	Styraceae Type II	26–33	13–17	21–25	13–16	1.7–2.7	–	–	–	–	–
34.	Styraceae Type III	21–24	16–17	21–28	20–21	3.1–4.8	–	–	–	–	–
35.	Styraceae Type IV	26–29	22–23	27–29	27–28	3–4	–	–	–	–	–
36.	Styraceae Type V	23–24	17–18	26–29	20–21	3.4–4.3	–	–	–	–	–
37.	*Symplocos* (Sympolaceae)	29–38	15–16	30-38	21–22	1.7–2.6	–	–	–	–	–
38.	Thymelaeaceae	25–26	17–18	23–26	16–17	4.2-5.8	–	–	–	–	–
39.	Ulmaceae (*Cedrelospermum*)	23–26	18–19	23–24	15–36	1.3–2.1	–	–	–	–	–
40.	Ephedraceae	23–25	13–14	27–45	35–16	1.3–2.1	–	–	–	–	–
41.	Pinaceae (*Cathya*)	55–59	45–51	22–25	20–23	1.7–2.5	–	–	–	–	–
42.	Trilete spore	33–43	26–28	–	–	2.7–4.1	–	–	–	–	–

Table 7. Qualitative features of fossil plants pollen from Insect limestone, United Kingdom.

S.No	Taxon	Pollen outline in equatorial View	Pollen outline in polar view	Number of colpi/pori	Exine sculpture (LM)	Exine Sculpture (SEM)	Suprasculpture (SEM)
1.	**Chloranthaceae**	elliptical	-	Monocolpate	Reticulate	Microreticulate to reticulate	Nano echinate
2.	Typhaceae	Circular	Circular	-	Reticulate	Microreticulate to reticulate	Nano echinate
3.	Altingiaceae (*Liquidambar*)	Circular	Circular	Pantaporate	Reticulate	Reticulate-perforate	-
4.	Aquifoliaceae **(*llex*)**	Elliptical	Trilobate	Tricolpate	Baculate	Baculate-clavate	Micro striate
5.	Buxaceae (*Buxus*)	Circular	-	-	Reticulate	Microreticulate	Nano echinate
6.	Caprifoliaceae (*Viburnum*)	Elliptical	Lobate	Tricolpate	Reticulate	Microreticulate	-
7.	Nyssaceae (Type I)	Circular	Triangular	Tricolpate	Scarbate-reticulate	Reticulate and perforate-fossulate	-
8.	Nyssaceae (Type II)	Elliptical	-	Tricolpate	Scarbate	Perforate-fossulate	-
9.	Ericaceae (Type I)	Quadrangular	-	Tricolporate	Scarbate	Granulate-fossulate	Nano echinate
10.	Ericaceae (Type II)	Quadrangular	Lobate	Tricolporate	Scarbate	Areolate-verrucate	Areolate-verrucate
11.	Ericaceae (Type III)	Quadrangular	-	Tricolporate	Scarbate	Areolate-verrucate	Areolate-verrucate
12.	Eucommiaceae (Eucommia)	Elliptical	Trilobate	Tricolporate	Psilate	Psilate-regulate	-
13.	Euphorbiaceae	Elliptical	Lobate	Tricolporate	reticulate-perforate	Perforate-microreticulate	-
14.	Fagaceae (Castaneoideae)	Elliptical	-	Tricolporate	Psilate	Psilate-regulate	Fossulate
15.	*Quercus* Type I	Elliptical	Lobate	Tricolporate	Scabrate-perforate	Verrucate-fossulate	-
16.	*Quercus* Type II	Elliptical	-	Tricolporate	Scabrate-perforate	Verrucate-fossulate	-

17.	Hamelidaceae	Elliptical	-	Tricolpate	Reticulate	Micro-reticulate-fossulate	Nano echinate
18.	Juglandaceae (*Carya*)	Elliptical	Circular- triangular	-	Psilate	Granulate-nanorugulate	-
19.	Juglandaceae Type I	Elliptical	Triangular	-	Psilate	Granulate-nanorugulate	-
20.	Loranthaceae	-	Triangular	Tricolpate	Psilate	Microbaculate	-
21.	Malvaceae (Tilioideae)	Elliptical	Circular	Tripoarte	Scabrate-perforate	Nanoreticulate-perforate	-
22.	Myricaceae (*Myrica*)	Elliptical	Triangular	-	Psilate	Scarbate-miroechinate	-
23.	Oleaceae Ttpe I	Circular-elliptical	Lobate	Tricolporate	Reticulate	Reticulate-fossulate	-
24.	Oleaceae Ttpe II	Circular-elliptical	-	Tricolporate	Reticulate	Microreticulate-reticulate	Nano echinate
25.	Platanus I	Circular-elliptical	Lobate	Tricolporate	Scabrate-reticulate	Microreticulate-reticulate	-
26.	*Platanus* II	Circular-elliptical	-	Tricolporate	Scabrate-reticulate	Microreticulate-reticulate	-
27.	Rosaceae	Elliptical	-	Tricolporate	Psilate	Striate	-
28.	Rutaceae	Elliptical	Lobate	Tricolpate	Reticulate	Reticulate-striatoreticulate-	-
29.	Salicaceae (*Salix*)	Elliptical	Lobate	Tricolpate	Reticulate	Microreticulate-reticulate	-
30.	Santalaceae Type I	Elliptical	Tribobate	Tricolpate	Psilate-echinate	Psilate-echinate	-
31.	Santalaceae Type II	Elliptical	Lobate	Tricolpate	Psilate-echinate	Psilate-echinate	-
32.	Styraceae Type I	-	Triangular	Tricolporte	Psilate-scabrate	Perforate-aerolate	-

33.	Styraceae Type II	-	Triangular	Tricolporte	Psilate-scabrate	Perforate-aerolate	-
34.	Styraceae Type III	-	Triangular	Tricolporte	Psilate-scabrate	Perforate-aerolate	-
35.	Styraceae Type IV	-	Triangular	Tricolporte	Psilate-scabrate	Perforate-aerolate	-
36.	Styraceae Type V	-	Triangular	Tricolporte	Psilate-scabrate	Perforate-aerolate	-
37.	*Symplocos* (Sympolaceae)	Elliptical	-	Tricolpate	Scabrate	Gemmate-verrucate	-
38.	Thymelaeaceae	Circular	-	-	Psilate-echinate	Psilate-echinate	-
39.	Ulmaceae (*Cedrelospermum)*	Elliptical irregular		Tetra-pantaporate	Psilate	Verrucate-granulate	Granulate
40.	Ephedraceae	Lobate	Elliptical	-	Psilate	Scabrate-Plicate	-
41.	Pinaceae (*Cathya)*	Elliptical	-	-	Scabrate	Verrucate-perforate	-
42.	Trilete spore	Elliptical	Triangular	-	Scabrate	Scabrate	-

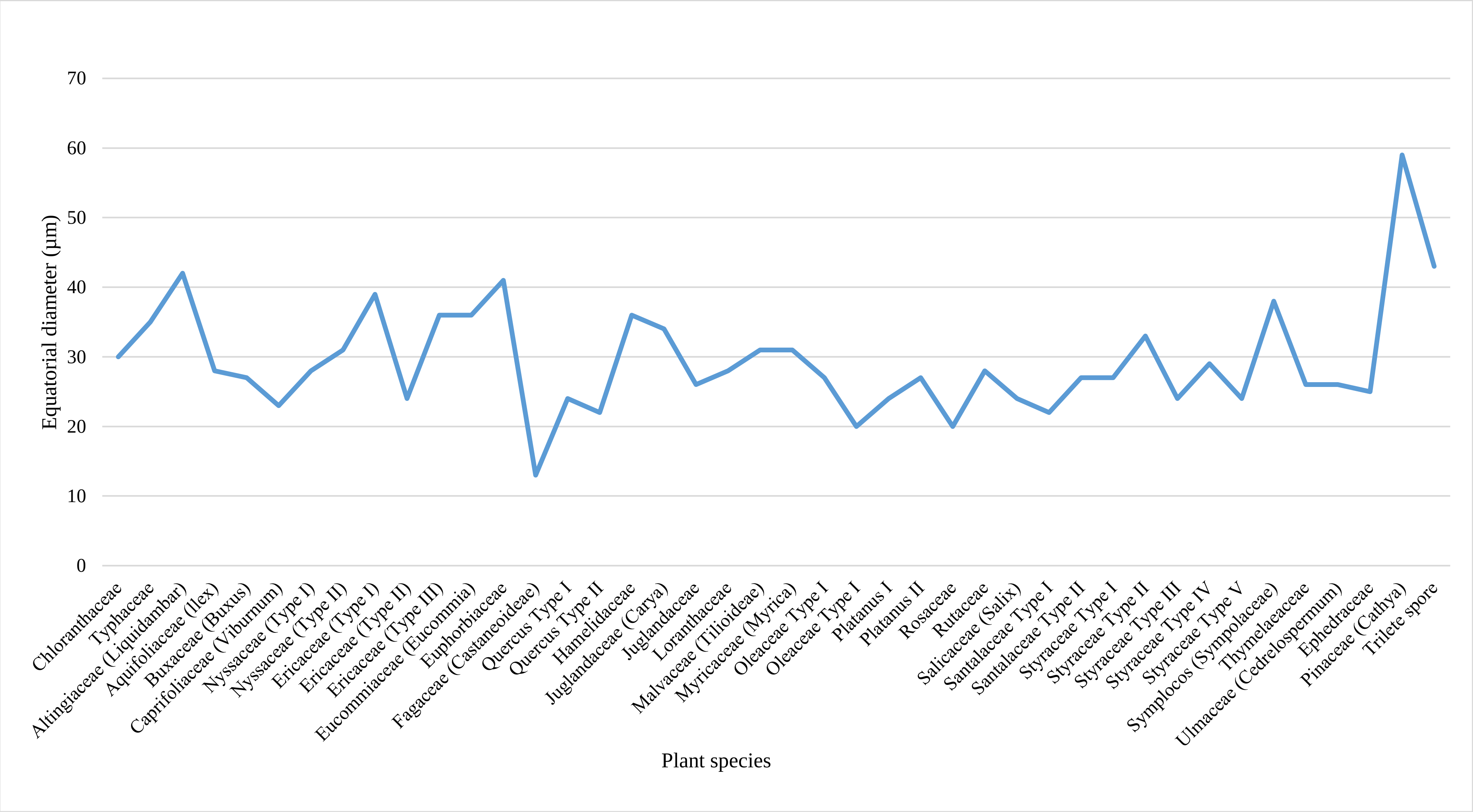

Figure 10. Variations in equatorial diameter among pollen grains of Eocene, Insect Limestone, United Kingdom, using light microscopy.

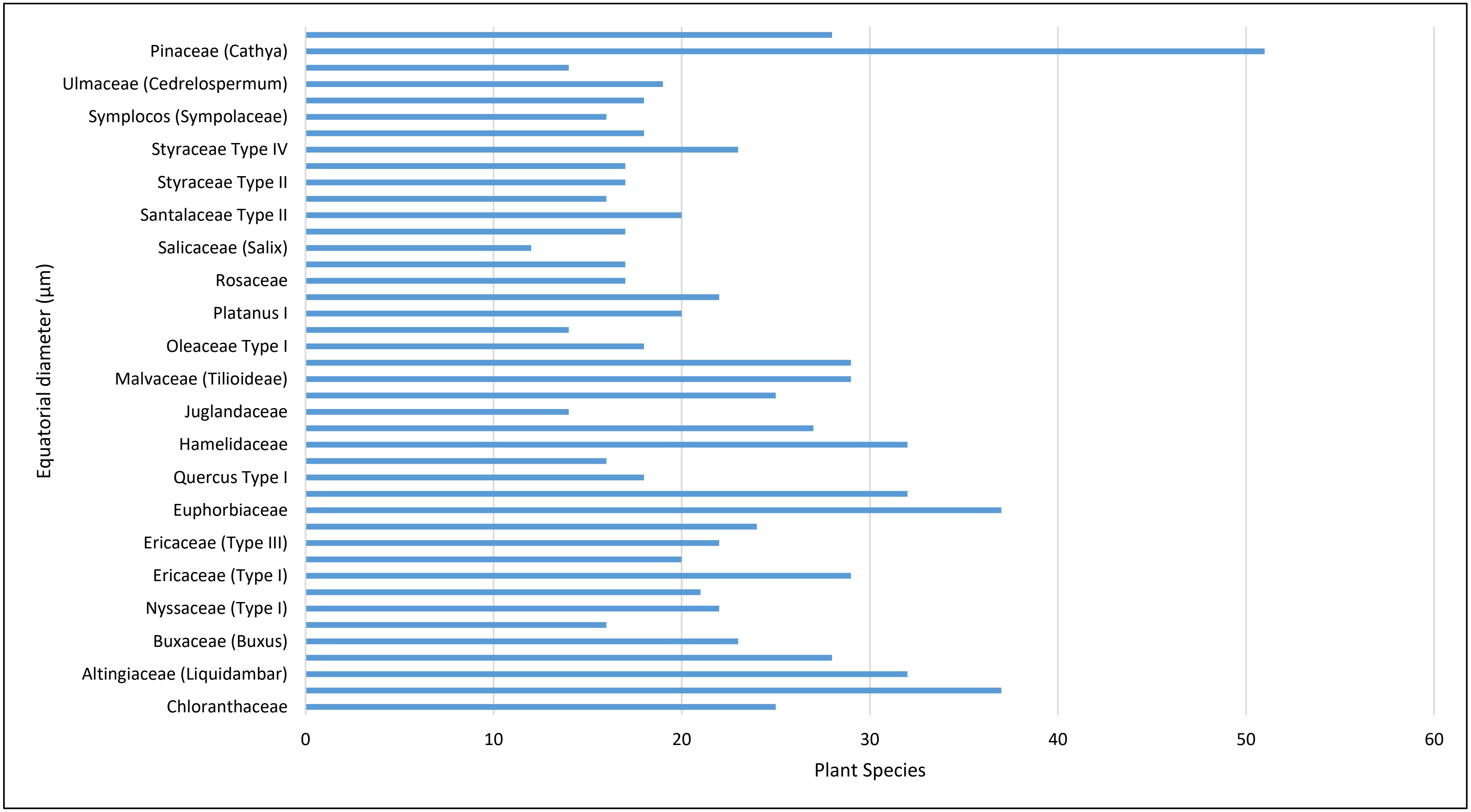

Figure 11. Variations in equatorial diameter among pollen grains of Eocene, Insect Limestone, United Kingdom, using scanning electron microscopy.

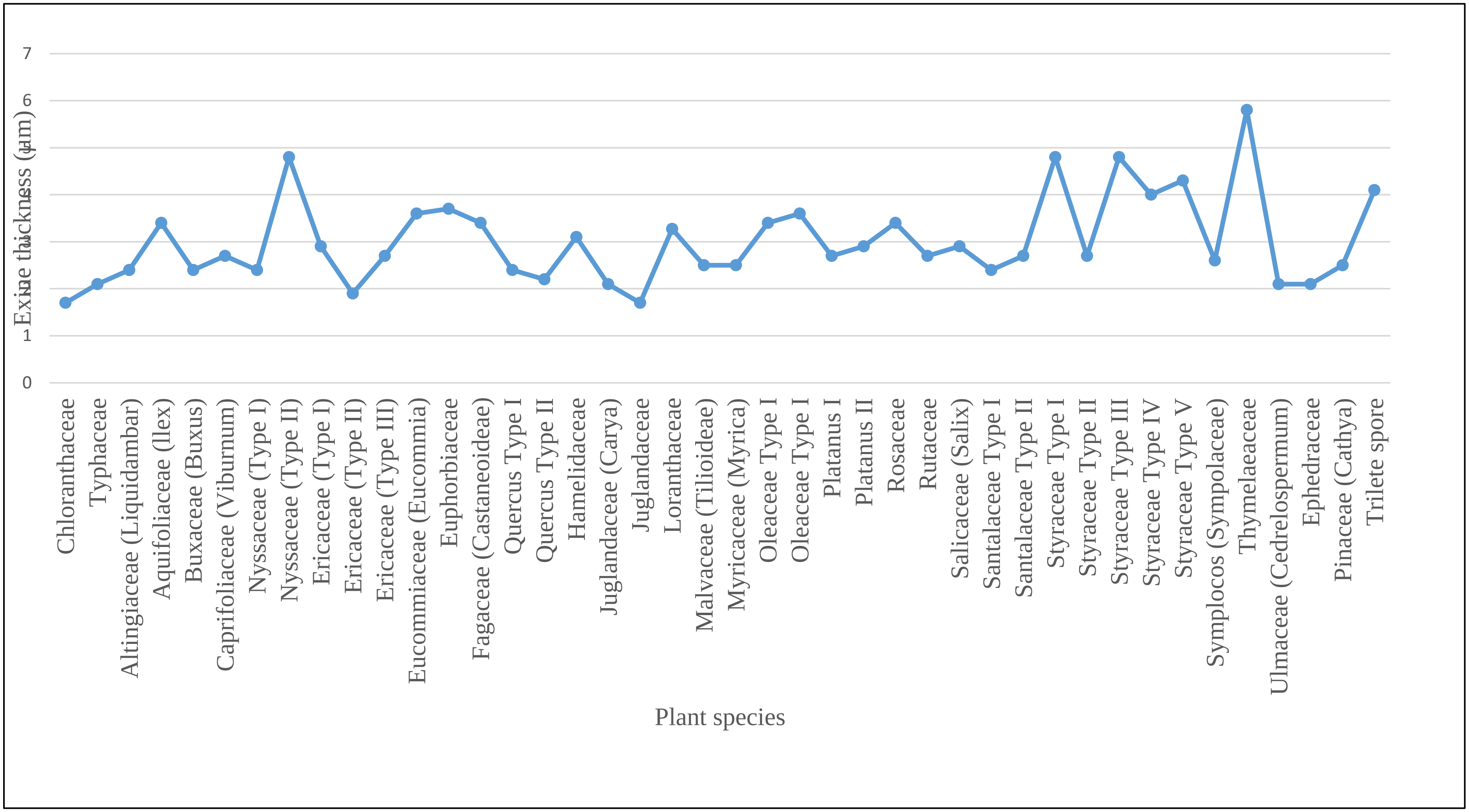

Figure 12. Variations in values of exine thickness in pollen grains of Eocene, Insect Limestone, United Kingdom, using light microscopy.

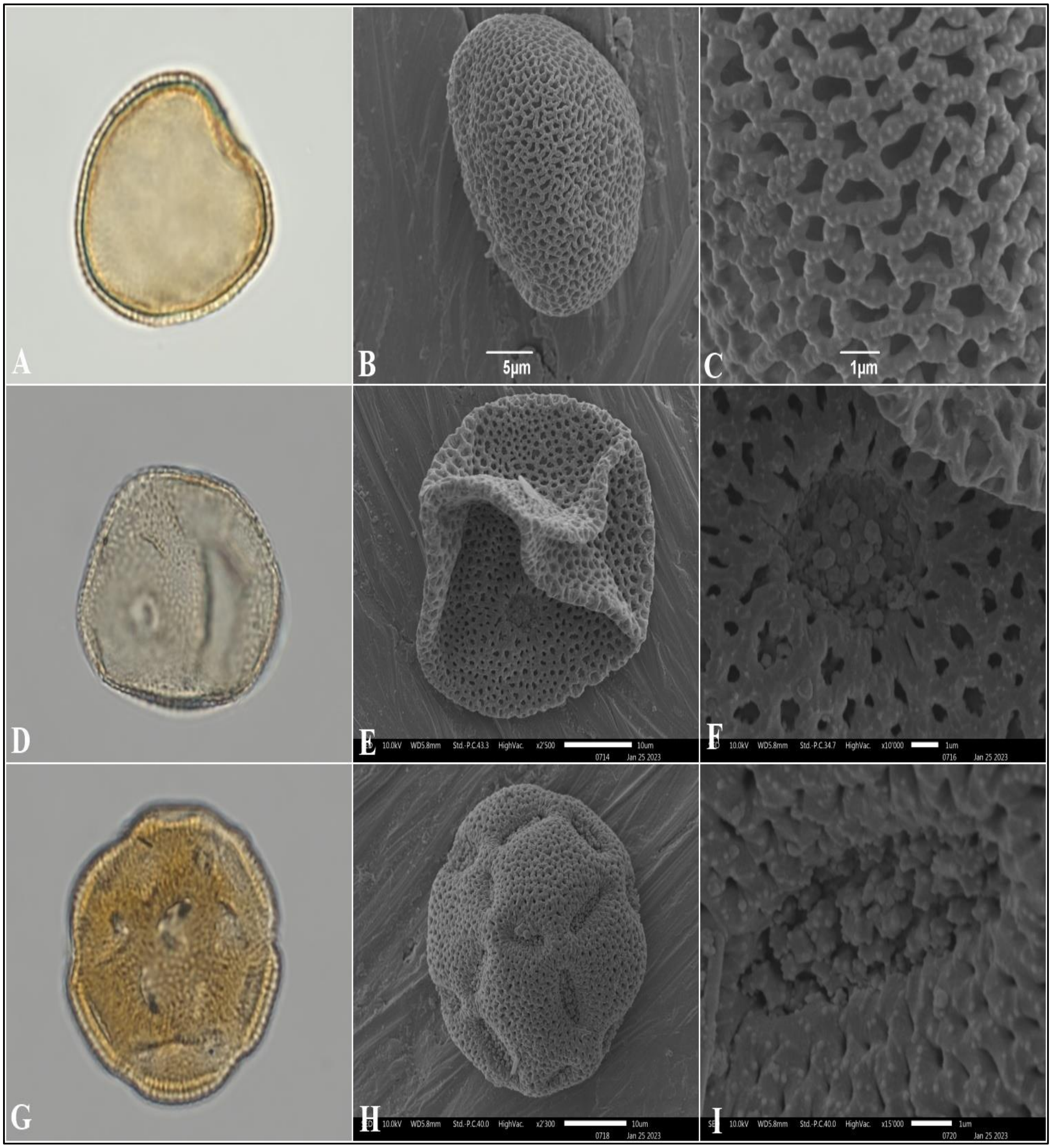

Plate 24. LM & SEM pollen photographs. A-C. Chloranthaceae; D-F. Typhaceae; G-I. Altingiaceae (*Liquidambar*)

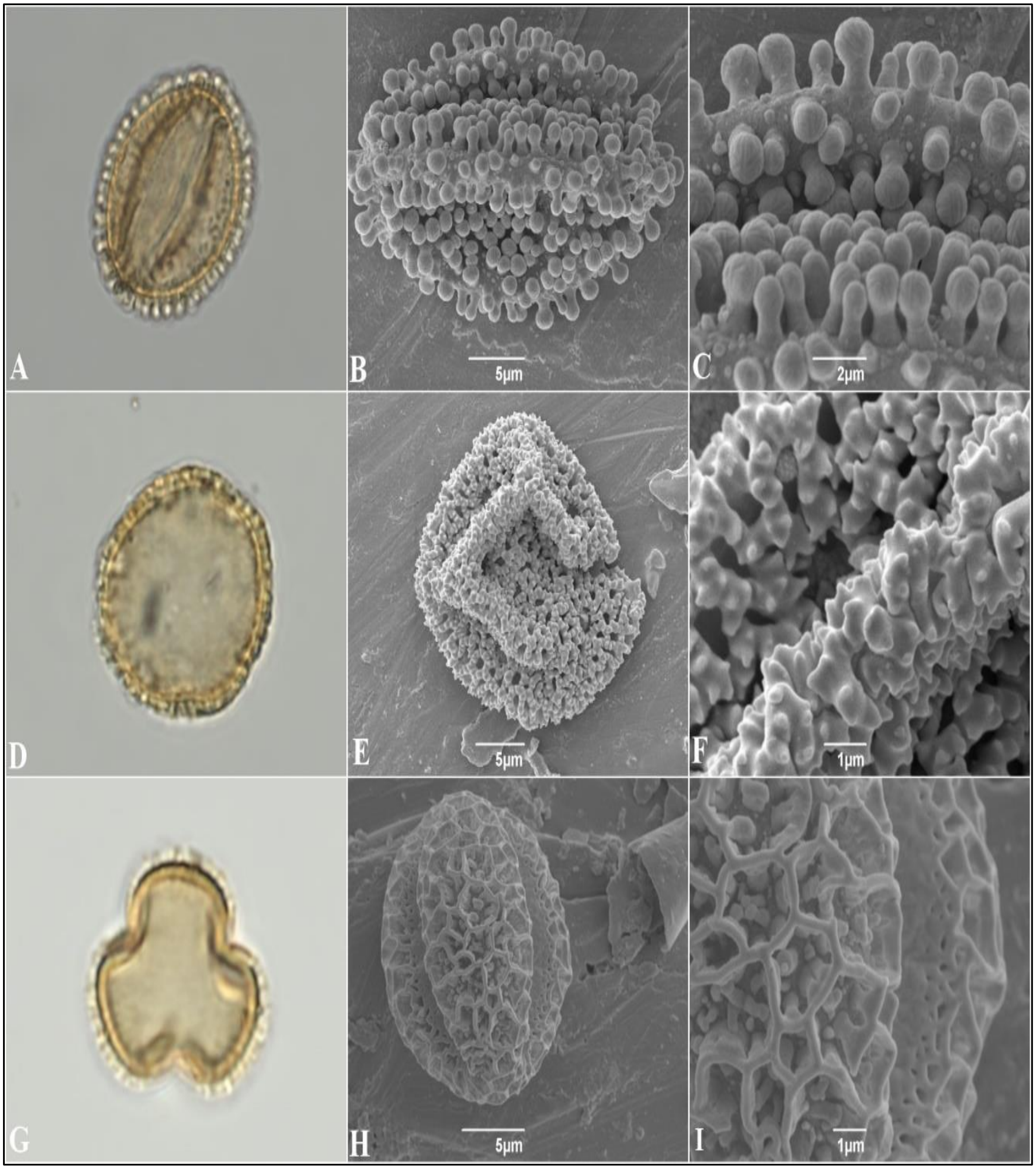

Plate 25. LM & SEM pollen photographs. A-C. Aquifoliaceae (*llex*) D-F. Buxaceae (Buxus); G-I. Caprifoliaceae (*Viburnum*)

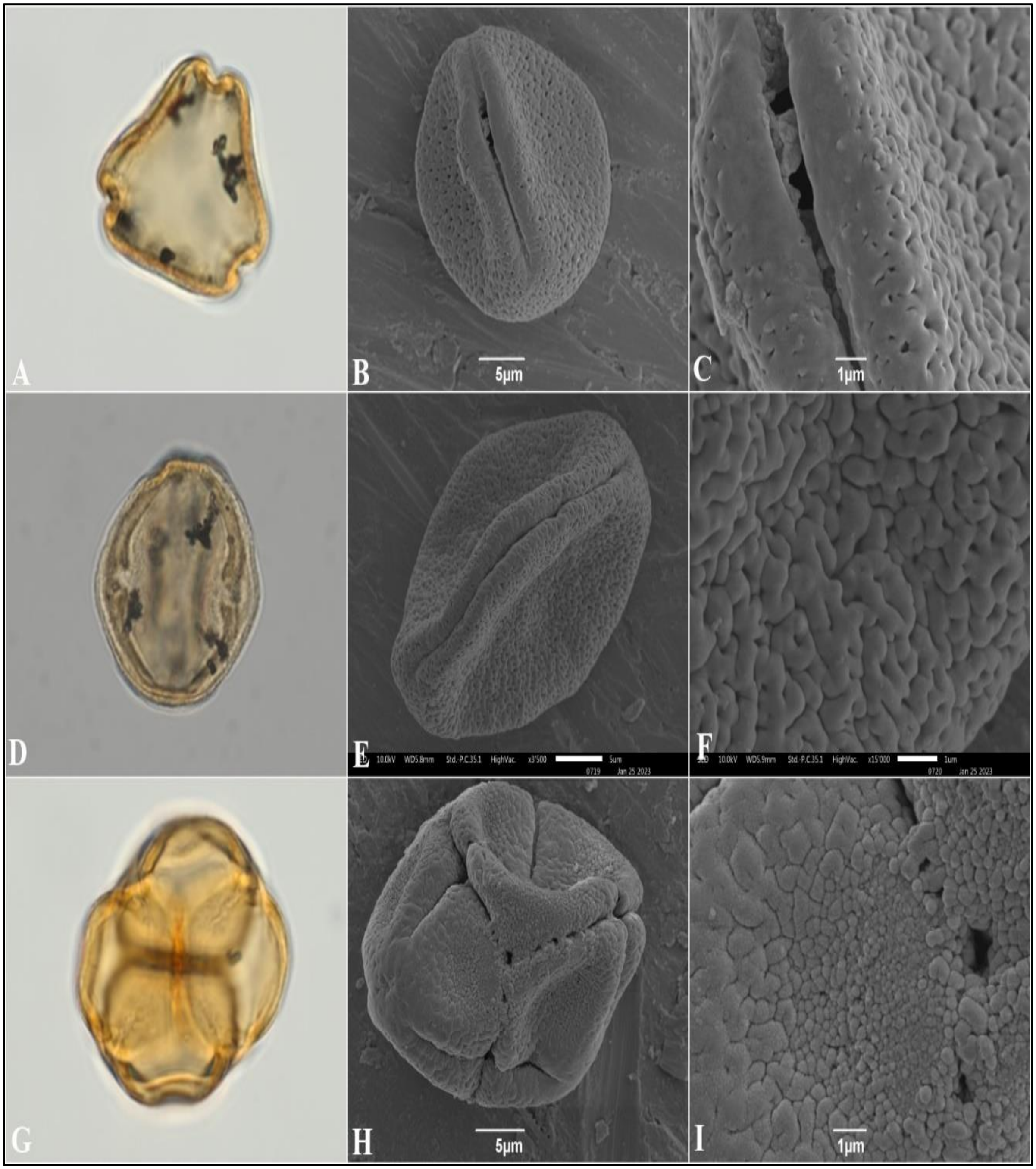

Plate 26. LM & SEM pollen photographs. A-F. Nyssaceae; G-I. Ericaceae

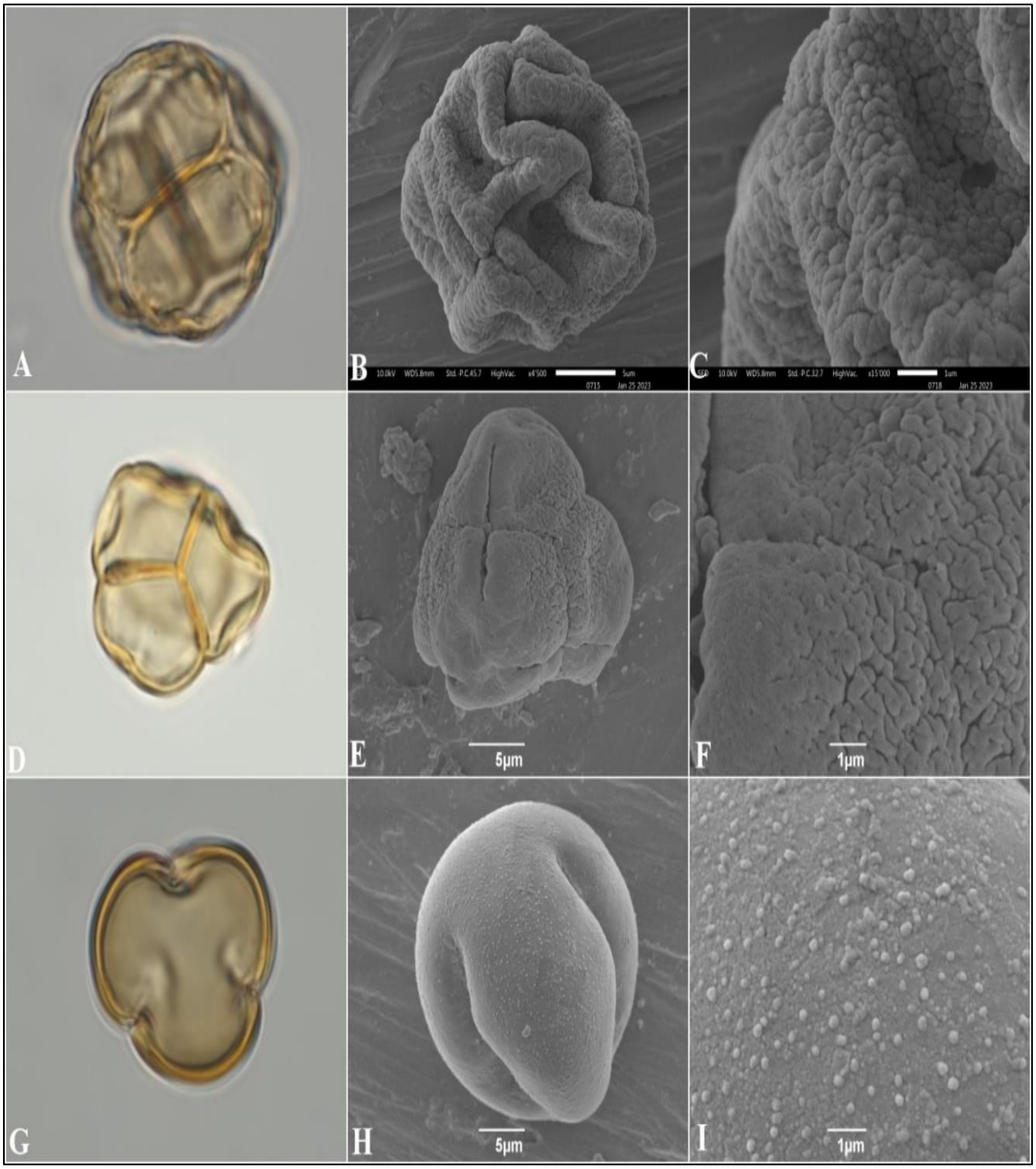

Plate 27. LM & SEM pollen photographs. A-F. Ericaceae; G-I. Eucommiaceae (*Eucommia*)

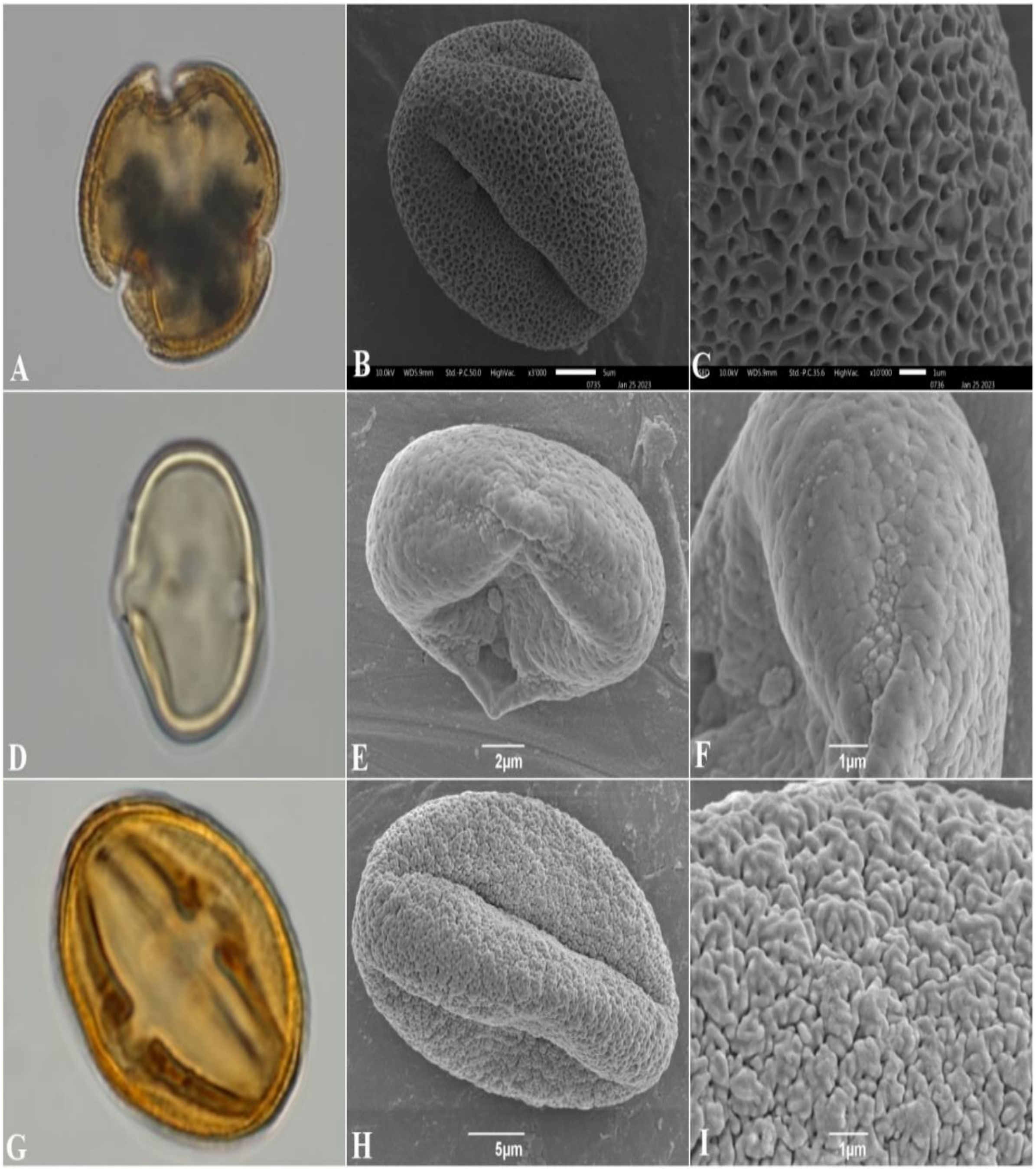

Plate 28. LM & SEM pollen photographs. A-C. Euphorbiaceae; E-G. Fagaceae (Castaneoideae); H-I. *Quercus* Type I

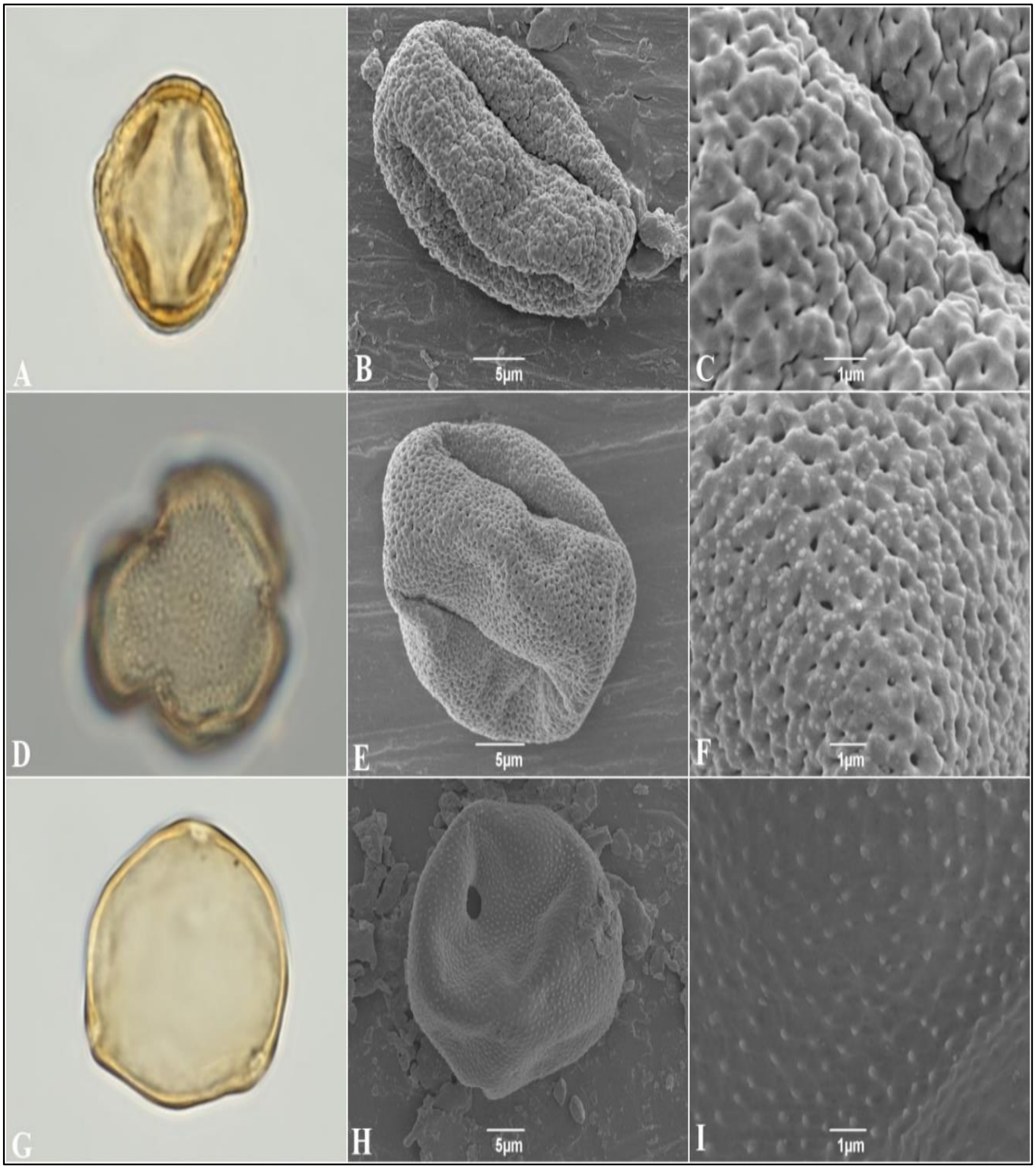

Plate 29. LM & SEM pollen photographs. A-C. *Quercus* Type II; D-F. Hamamelidaceae H-I. Juglandaceae (*Carya*)

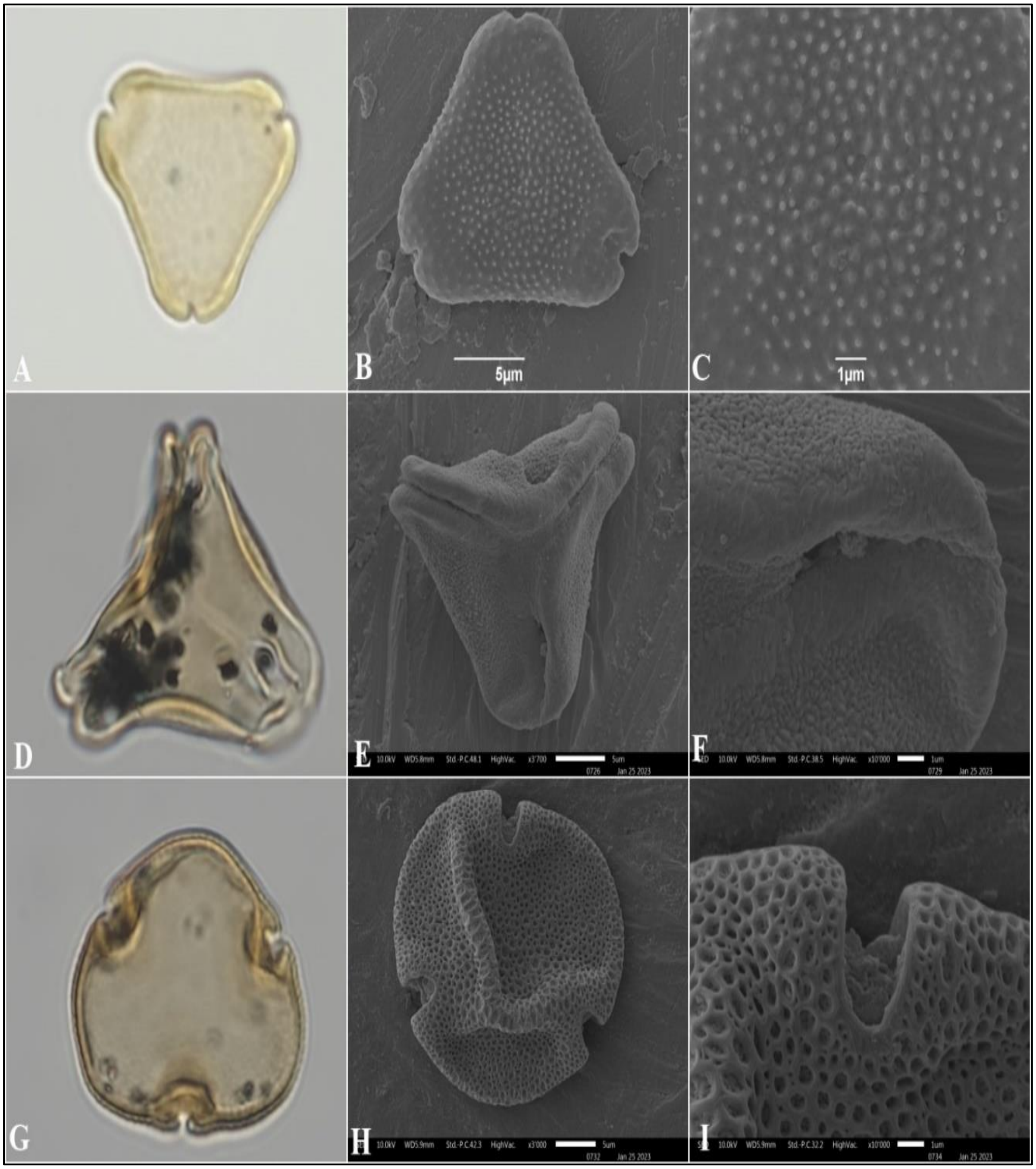

Plate 30. LM & SEM pollen photographs. A-C. Juglandaceae Type I; D-F. Loranthaceae H-I. Malvaceae (Tilioideae)

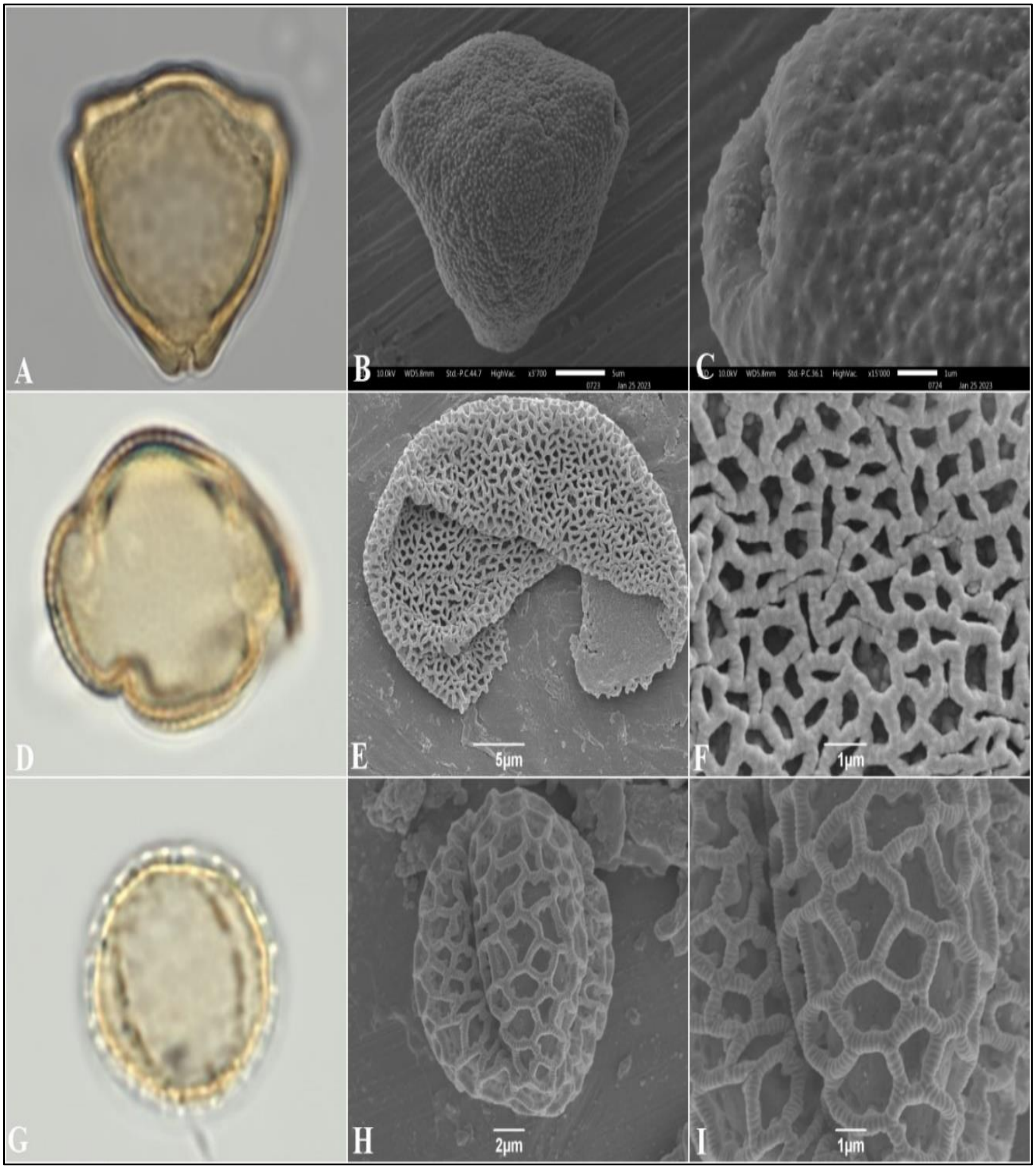

Plate 31. LM & SEM pollen photographs. A-C. Myricaceae (*Myrica*); D-I. Oleaceae

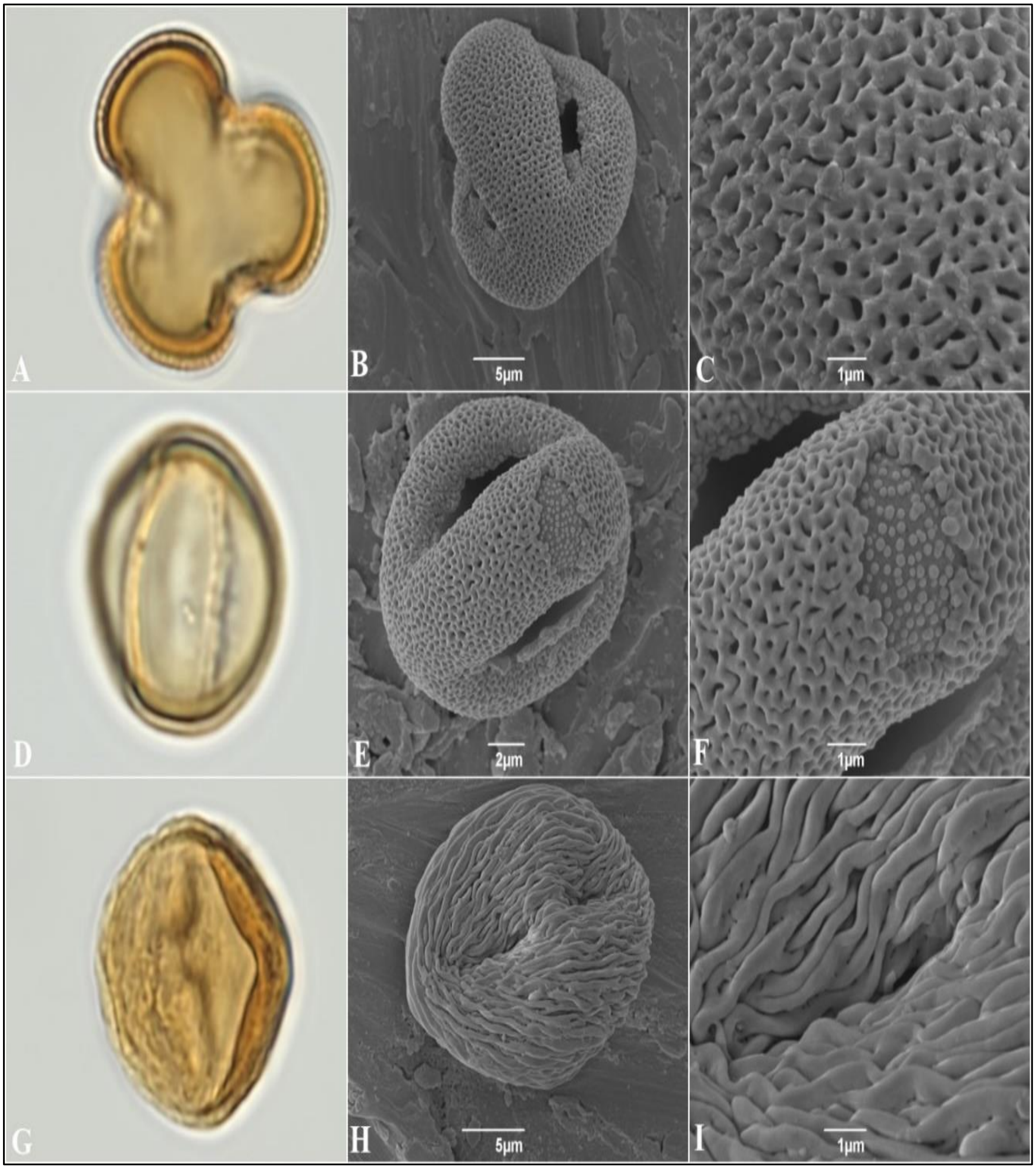

Plate 32. LM & SEM pollen photographs. A-C. Platanaceae (*Platanus* I); D-F. Platanaceae (*Platanus* II); G-I. Rosaceae

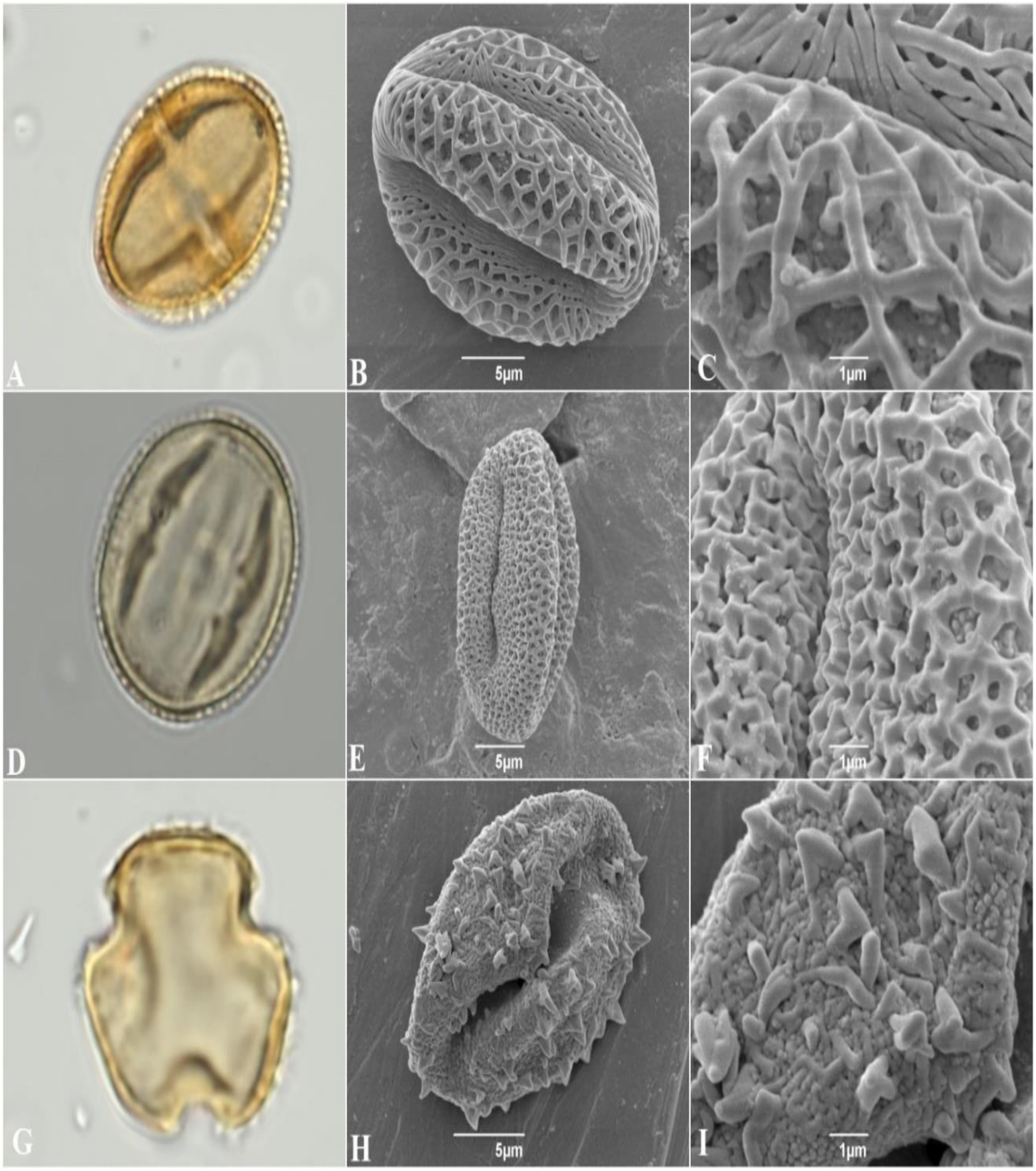

Plate 33. A-C. Rutaceae; D-F. Salicaceae (*Salix*); G-I. Santalaceae Type I

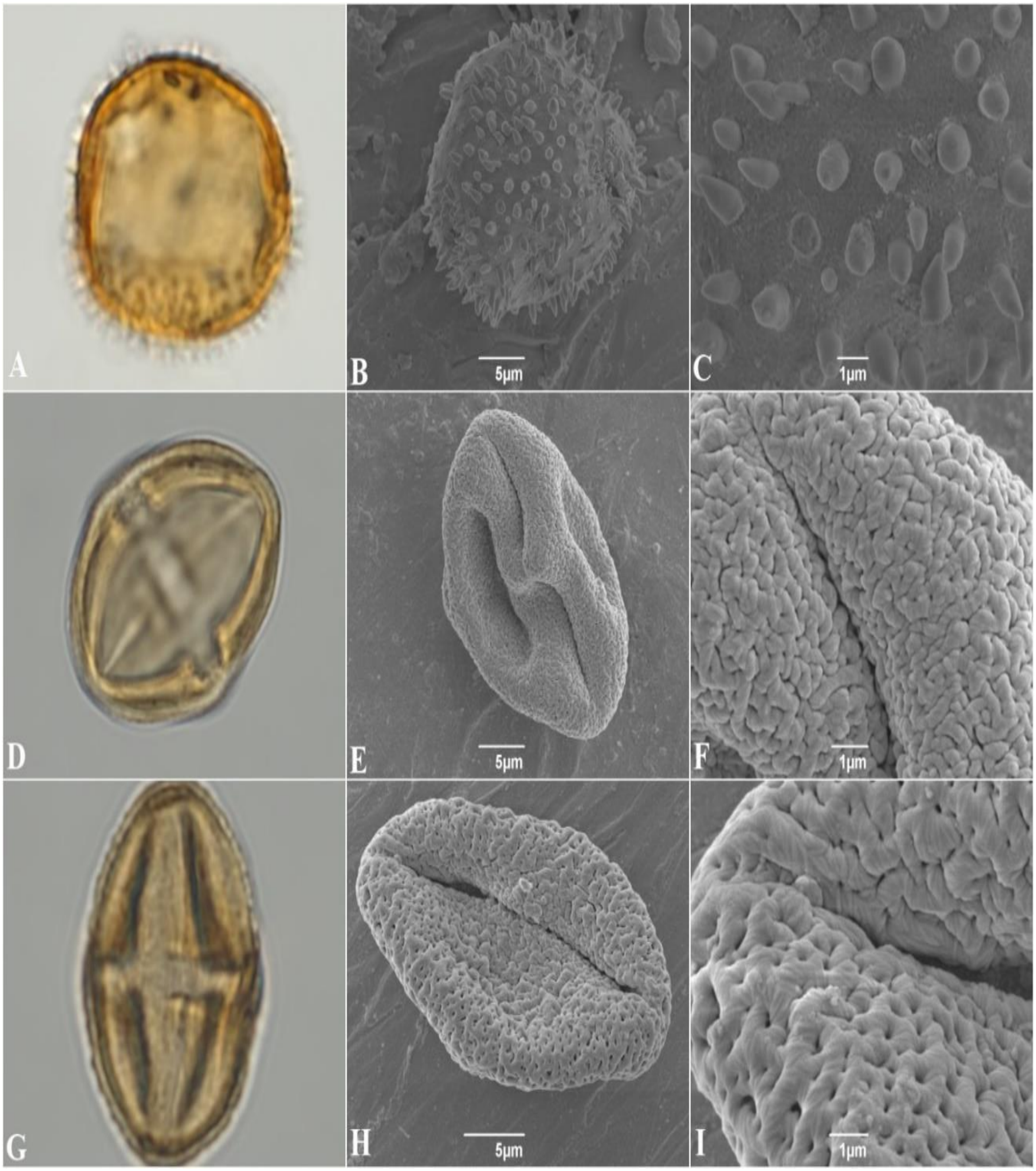

Plate 34. LM & SEM pollen photographs. A-C. Santalaceae Type II; D-I. Styraceae

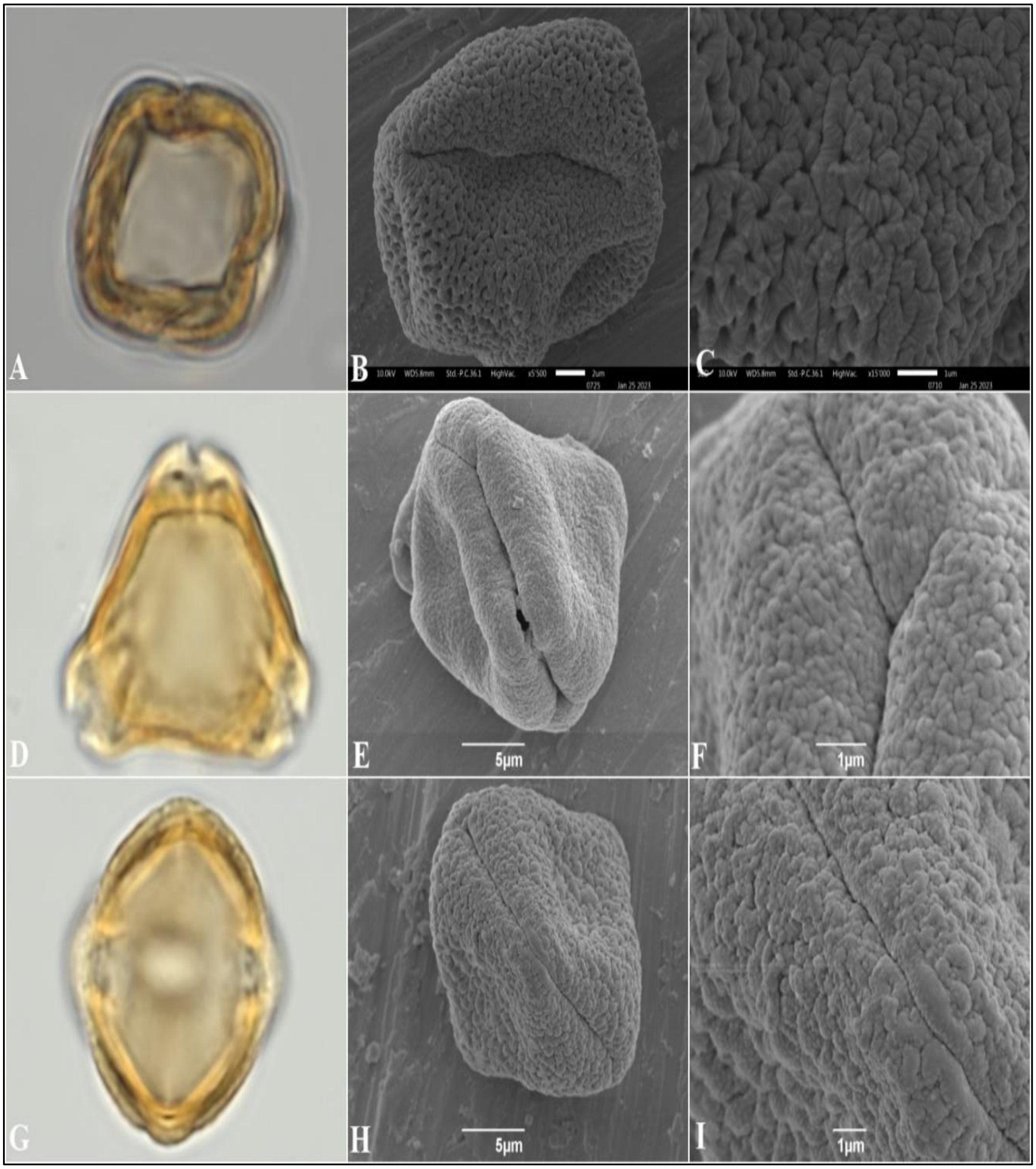

Plate 35. LM & SEM pollen photographs. A- I**.** Styraceae

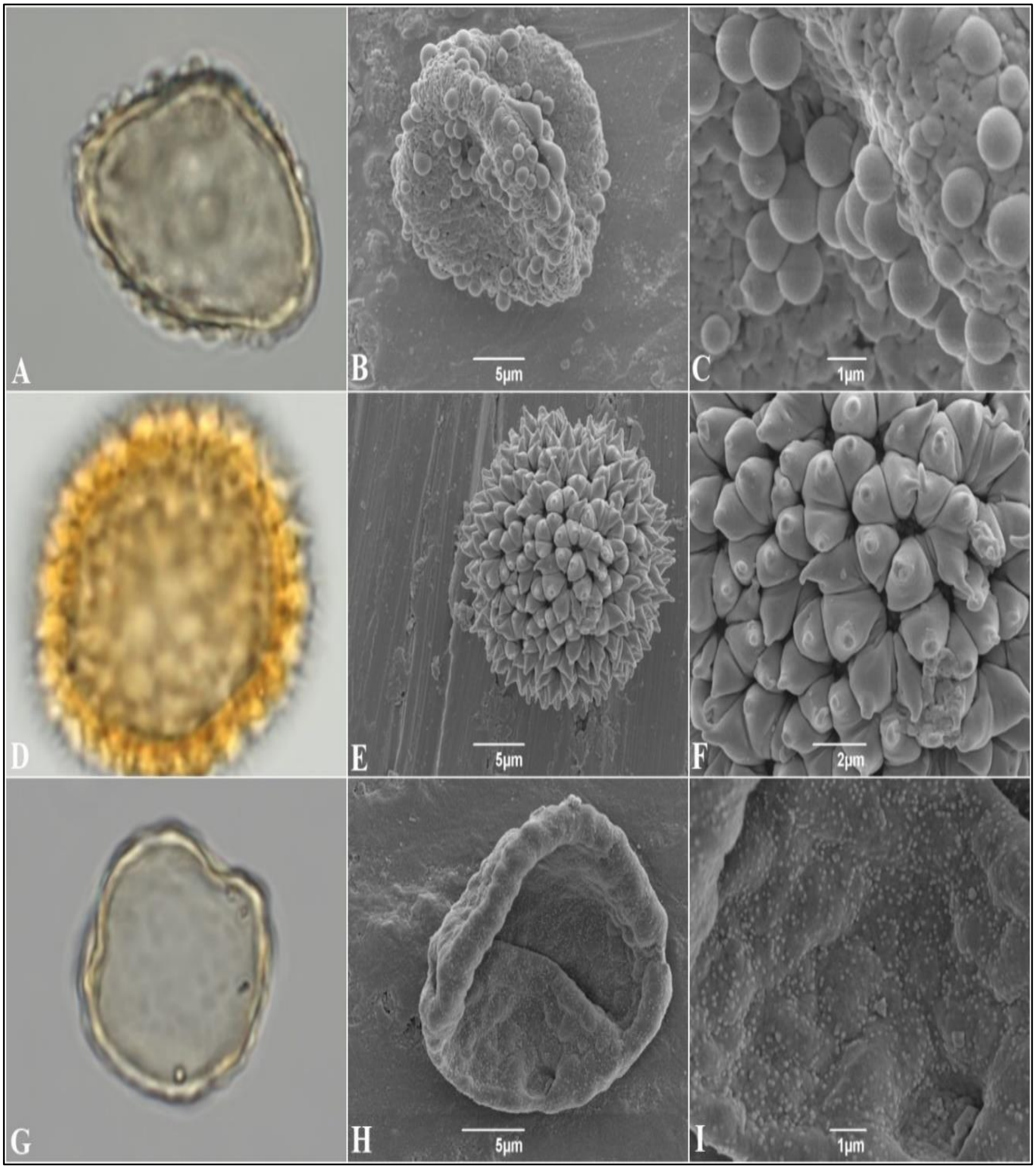

Plate 36. A-C. *Symplocos* (Sympolaceae); D-F. Thymelaeaceae; G-I. Ulmaceae (*Cedrelospermum)*

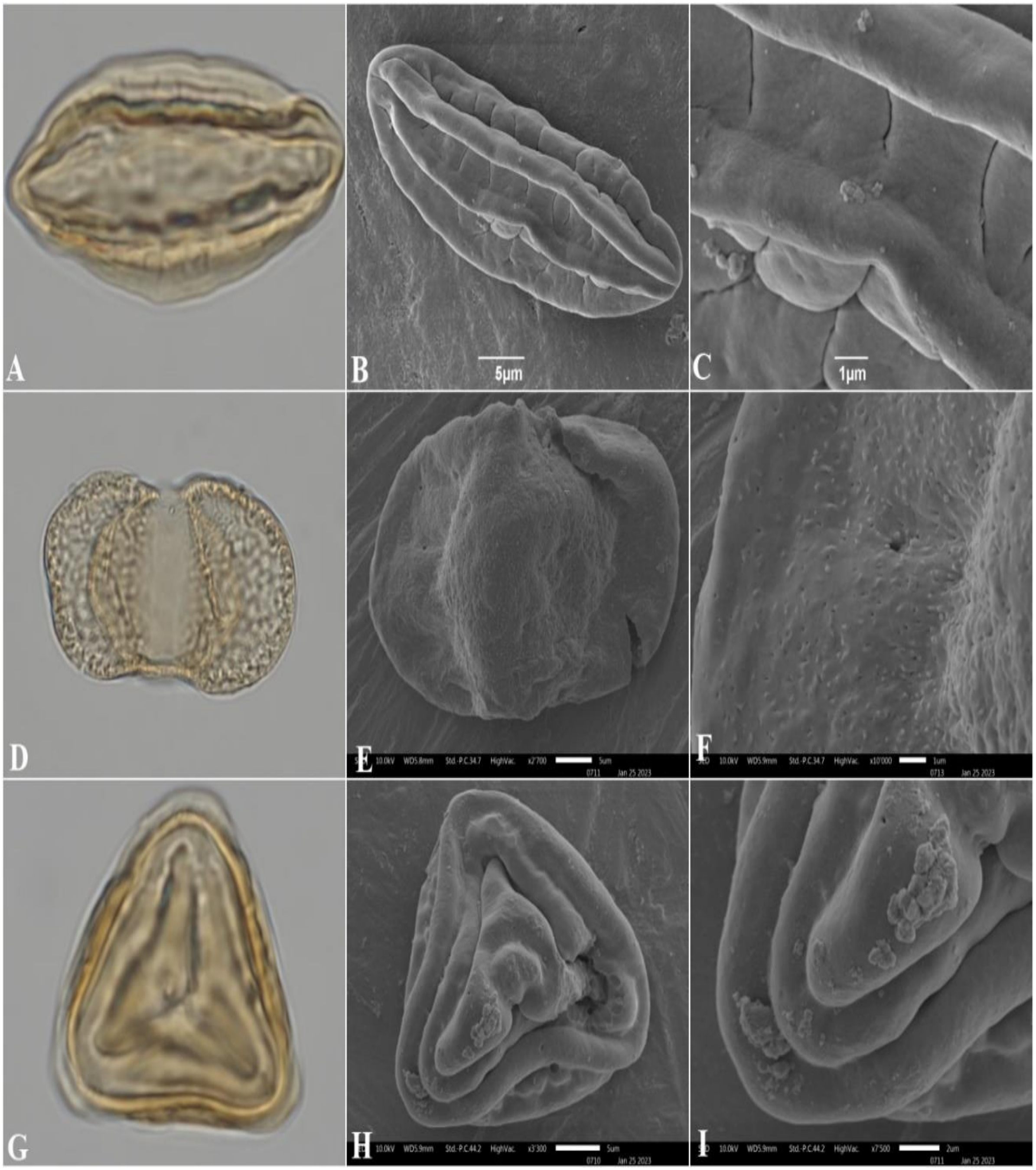

Plate 37. LM & SEM photographs. A-C. Ephedraceae; D-F. Pinaceae (*Cathya*); G-I. Trilete spore

SECTION: III

Paleocene, Patala Formation

3.3.1. Angiosperms monocots

a) Araceae

Pollen, monad, outline elliptical in equatorial view, equatorial diameter 30-31 µm (LM), 29-30 µm (SEM), polar axis 39-40 m (LM), 35-36 µm (SEM), exine thickness 4.2-5.3 µm (LM), pollen grains are unequal, containing two halves, exine ornamentation psilate (LM) foveolate, micro-perforate, and micro-perforations also found outside the pollen sculpture (SEM) as shown in (Table 8, Plate 38).

Remarks: The palynological record of Araceae was documented in Germany having micro-perforate to micro-reticulate exine ornamentation and resembles our results (Hesse and Zetter, 2007).

b) Aracaceae

Type I

Pollen, monad, outline elongated, equatorial diameter 17–18 µm (LM), 15–16 µm (SEM), polar axis 31–32 m (LM), 28–29 µm (SEM), exine thickness 1.1–1.4 µm (LM), exine ornamentation psilate (LM), fossulate-perforate (SEM), monosulcate with broadly elliptical to asymmetrical (SEM) as shown in (Table 8, Plate 38).

Type II

Pollen, monad, outline elongated, polar axis 34–35 m (LM), 33–34 µm (SEM), equatorial diameter 18–19 µm (LM), 19–20 µm (SEM), exine thickness 1.2–1.5 µm (LM), exine ornamentation psilate (LM), fossulate-perforate (SEM), monosulcate with broadly elliptical to asymmetrical (SEM) as shown in (Table 8, Plate 38).

Type III

Pollen, monad, outline trilobate in polar view, elliptic in equatorial view, equatorial diameter 20–21 µm (LM), 17–18 µm (SEM), polar axis 35-36 m (LM), 31-32 µm (SEM), exine thickness 1.3–1.6 µm (LM), isopolar, tricolpate, colpi long: exine ornamentation baculate, (LM & SEM), clavae with microstriate suprasculpture, aperture membrane clavate, sexine and nexine indiscernible (SEM) as shown in (Table 8, Plate 39).

Type IV

Pollen, monad, heteropolar, outline circular to elliptical, perforated (SEM), equatorial diameter 19–20 µm (LM), 17–18 µm (SEM), polar axis 35–36 m (LM), 31–32 µm (SEM), exine thickness 1.1–1.4 µm (LM), P/E ratio oblate, exine ornamentation psilate-scabrate (LM), nexine thicker than sexine as shown in (Table 8, Plate 39).

Type V

Pollen, monad, outline elliptical, P/E ratio oblate, polar axis 43–44 (LM), 40–41 µm (SEM), equatorial diameter 17–18 µm (LM), 16–17 µm (SEM), exine thickness 1.3–1.5 µm (LM), mononucleate, sulcus prominent, broader in middle and narrow towards ends, sulcus length 37-38 µm, width 1.4–4.3 µm (LM); columellae obscure; exine ornamentation psilate (LM), perforate (SEM) as shown in (Table 8, Plate 39).

Type VI

Pollen, monad, outline elliptical, P/E ratio oblate, polar axis 37–38 (LM), 36–37 µm (SEM), equatorial diameter 15–16 µm (LM), 16–17 µm (SEM), exine thickness 1.6–2.1 µm (LM), exine ornamentation psilate (LM), psilate-scabrate (SEM), perforate (SEM), heteropolar, P/E ratio oblate as shown in (Table 8, Plate 40).

Type VII

Pollen, monad, outline elliptical, P/E ratio oblate, polar axis 33–34 µm (LM), 31–32 µm (SEM), equatorial diameter 23–24 µm (LM), 20–21 µm (SEM), exine thickness 1.1–1.4 µm (LM), exine ornamentation psilate (LM), perforate (SEM), heteropolar, P/E ratio oblate as shown in (Table 8, Plate 40).

Type VIII

Pollen, monad, outline elongated, polar axis 27–28 µm (LM), 22–23 µm (SEM), equatorial diameter 15–16 µm (LM), 11–12 µm (SEM), exine thickness 1.4–1.8 µm (LM), P/E ratio oblate, exine ornamentation psilate-scabrate (LM), foveolate-perforate (SEM) as shown in (Table 8, Plate 40).

Type IX

Pollen, monad, outline elliptical, P/E ratio oblate, polar axis 33–34 µm (LM), 30–31 µm (SEM), equatorial diameter 22–23 µm (LM), 19–20 µm (SEM), exine thickness 1.1–1.5 µm (LM), P/E ratio oblate, exine ornamentation psilate-scabrate (LM), foveolate to perforate (SEM) as shown in (Table 8, Plate 41).

Remarks: *Arecaceae* pollen grains were variable in size. The arrangements in the sulcus are quite different from each other. The fossil records of the *Arecaceae* have been determined from the Paleocene in the Lingfeng Formation and from the Oligocene in the South China Sea (Huang *et al*., 2021).

c) Poaceae

Pollen, monad, outline elliptic in equatorial view, polar axis 47–48 µm (LM), 33–34 µm (SEM), equatorial diameter, 31–32 µm (LM), 29–30 µm (SEM), exine thickness 2.4–2.9 µm (LM), P/E ratio oblate, nexine thinner than sexine, ornamentation scabrate (LM), fossulate, and perforate (SEM) as shown in (Table 8, Plate 41).

Remarks: Many fossil phragmites have been investigated in Eurasia. Poaceae have aerolated pollen, which corresponds to the current fossil pollen of Poaceae (Köhler and Lange, 1979).

3.3.2. Gymnosperms

Cycadaceae

Type I

Pollen, monad, outline elliptical, equatorial diameter 15–16 µm (LM), 12–13 µm (SEM), exine thickness 1.3–1.6 µm (LM), polar axis 27–28 µm (LM), 19–20 µm (SEM), sexine and nexine thickness are equal, ornamentation psilate (LM), foveolate-perforate (SEM) as shown in (Table 8, Plate 41).

Type II

Pollen, monad shape elliptical in equatorial view, monosulcate, sulcus prominent, broader in the center and narrow towards the ends, sulcus length 16–17 µm width 3–3 µm (SEM); equatorial diameter 17–18 µm (LM), 16–17 µm (SEM), polar axis 35–36 µm (LM), 30–31 µm (SEM), exine thickness 2.1–2.5 µm (LM), ornamentation psilate (LM), foveolate-perforate (SEM) as shown in (Table 8, Plate 42).

Remarks: Pollen grains of the *Cycadales* exist in tropical forests and riparian paleoenvironments (Akkiraz *et al*., 2008). The species were spread globally in the Cenozoic regions, and the pollen grains are different morphologically from the others due to plicae and branched pseudosulci (Smith *et al*., 2020).

3.3.3. Angiosperms Dicots

a) Rosaceae

Pollen, monad, outline elliptical in equatorial view, equatorial diameter 23–24 µm (LM), 18–19 µm (SEM), polar axis 33–34 µm (LM), 32–33 µm (SEM), exine thickness 1.7–2 µm (LM), exine ornamentation psilate (LM), striate, perforate (SEM); striae oriented within groups, densely packed and branched (SEM) as shown in (Table 8, Plate 42).

Remarks: Pollen grains of the Rosaceae resemble in some features i.e., striate exine ornamentation to the extant fossils (c, 1981). It is entomophilous and grows in waterlogged soil and open habitats (eFlora, 2008).

b) Sapotaceae

Type I

Pollen, monad, polar axis 28–29 µm (LM), 26–27 µm (SEM), equatorial diameter 23–24 µm (LM), 21–22 µm (SEM), exine thickness 2.3–2.9 µm (LM), P/E prolate, Elliptical-circular in equatorial view, exine ornamentation psilate, tricolpate, sexine thicker than nexine, close-up view showing micro-verrucate and perforate sculpturing of mesocolpium (SEM), ornamentation psilate-scabrate (LM), microregulate-perforate (SEM) as shown in (Table 8, Plate 42).

Type II

Pollen, monad, elliptical-circular in equatorial view, equatorial diameter 19–20 µm (LM), 22–23 µm (SEM), polar axis 21–22 µm (LM), 24–25 µm (SEM), exine thickness 1.2–1.5 µm (LM), P/E ratio prolate, ornamentation psilate, tricolpate, sexine thicker than nexine, close-up view showing micro-verrucate, ornamentation psilate to scabrate (LM), microregulate-perforate (SEM) as shown in (Table 8, Plate 43).

Remarks: Sapotaceae members were entomophilous and exist along streams and lowland forests. The Sapotaceoidae pollenites genus is used for the fossil and extant species of Sapotaceae. The earliest record of the family was investigated in the Paleocene in Borneo, Europe, and America (Muller, 1981).

c) Anacardiaceae

Type I

Pollen, monad, outline elliptical, polar axis 20–21 µm (LM), 18–19 µm (SEM), tricolpate equatorial diameter 14–15 µm (LM), 12–13 µm (SEM), exine thickness 0.7–0.9 µm (LM exine ornamentation scabrate (LM), striated (SEM) as shown in (Table 8, Plate 43).

Type II

Pollen, monad, outline elliptical, polar axis 28–29 µm (LM), 16–17 µm (SEM), equatorial diameter 21–22 µm (LM), 13–14 µm (SEM), exine thickness 0.9–1.2 µm (LM), tricolpate, nexine thicker than sexine (LM), exine ornamentation scabrate (LM), microreticulate (SEM), muri crested by nanochini (SEM) as shown in (Table 8, Plate 43).

Remarks: Pollen have reticulated exine ornamentation having muri crested of nanoechini. These types of species are found in Mediterranean areas. Wodehouse (1933 reported *Rhoipites* as a fossil member of this family. *Tricolporopollenites pseudocingulum* has been investigated from Paleogene of Eurasia and North America in the Oligocene (Muller, 1981).

d) Cannabaceae

Pollen, monad, outline circular, exine thickness 1.0–1.4 µm (LM)), equatorial diameter 17–18 µm (LM), 15–16 µm (SEM), polar axis 19–20 µm (LM), 18–19 µm

(SEM), sexine thinner than nexine (SEM). P/E ratio prolate, exine ornamentation psilate (LM), perforate, and nanogemmate (SEM) as shown in (Table 8, Plate 44).

Remarks: In morphological features i.e. exine ornamentation, size, shape, outline, and pore configuration, the pollen grains resemble the *Cletis*. The species is anemophilous and is found in lowland and upland forests (eFlora, 2008).

e) Ebenaceae (*Diospyros*)

Type I

Pollen, monad, outline circular-elliptical, polar axis 32–33 µm (LM), equatorial diameter 18–19 µm (LM), 17–18 µm (SEM), 31–32 µm (SEM), exine thickness 0.9–1.7 µm (LM), exine ornamentation psilate (LM), regulate-perforate, aperture membrane granulates (SEM) as shown in (Table 8, Plate 44).

Type II

Pollen, monad, outline circular-elliptical, polar axis 19–20 µm (LM), 17–18 µm (SEM), equatorial diameter 18–19 µm (LM), 15–16 µm (SEM), exine thickness 1.1–2.4 µm (LM), exine ornamentation psilate (LM), regulate (SEM) as shown in (Table 8, Plate 44).

Remarks: In exine ornamentation and aperture configurations the *Diospyros* corresponds to the Its fossil pollen (Geeraerts *et al*., 2009). It is pollinated by insects and can exist in open habitats and forests.

f) Euphorbiaceae

Type I

Pollen, monad, outline elliptical, equatorial diameter 17–20 µm (LM), 22–24 µm (SEM), polar axis 25–30 µm (LM), 24-28 µm (SEM), exine thickness 1.1–2.4 µm (LM), tricolpate, exine ornamentation psilate (LM), verrucate-microverrucate, and perforate-granulate (SEM) as shown in (Table 8, Plate 45).

Type II

Pollen, monad, outline elliptical, equatorial diameter 21–22 µm (LM), 17–18 µm (SEM), polar axis 27–31 µm (LM), 25–26 µm (SEM), exine thickness 1.3–1.8 µm (LM), tricolpate, exine ornamentation psilate (LM), verrucate-microverrucate (SEM) as shown in (Table 8, Plate 45).

Type III

Pollen, monad, outline elliptical, equatorial diameter 17–20 µm (LM), 22–24 µm (SEM), exine thickness 1.3–2.2 µm (LM), tricolpate, exine ornamentation psilate (LM), reticulate (SEM) as shown in (Table 8, Plate 45).

Type IV

Pollen, monad, outline elliptical, equatorial diameter 18–19 µm (LM), 20–21 µm (SEM), polar axis 26–29 µm (LM), 23–25 µm (SEM), exine thickness 1.5–2.7 µm (LM), tricolpate, exine ornamentation psilate (LM), reticulate (SEM) as shown in (Table 8, Plate 46).

Remarks: Fossil members of the Euphorbiaceae are tricolpate and have been reported from the Cenozoic era of China. Zhi-Chen, 2014 reported fossil members of *Planotricolporites* from the Ganchaigou formation.

g) Fabaceae

Fabaceae Type I

Pollen, monad, outline circular, polar axis 27–28 µm (LM), 25–26 µm (SEM), equatorial diameter 25–26 µm (LM), 24–25 µm (SEM), exine thickness 0.9–1.3 µm (LM), sexine and nexine thickness are equal (SEM), exine ornamentation scabrate-reticulate (LM), reticulate (SEM) as shown in (Table 8, Plate 46).

Fabaceae Type II

Pollen, monad, spherical, equatorial diameter 21–22 µm (LM), 18–19 µm (SEM), polar axis 24–25 µm (LM), 21–22 µm (SEM), exine thickness 0.8–1 µm (LM), sexine and nexine thickness are equal (SEM), exine ornamentation scabrate reticulate (LM), reticulate (SEM) as shown in (Table 8, Plate 46).

Remrks: Morphological features of the pollen show reticulate, fossulate, and perforate exine ornamentation that corresponds to the many fossil and extant members of the Fabaceae (Hofmann, 2015).

h) Moraceae

Type I

Pollen, monad, outline circular, polar axis 13–18 µm (LM), 14–15 µm (SEM), equatorial diameter 13–16 µm (LM), 12–13 µm (SEM), exine thickness 0.7–0.9 µm (LM), sexine thinner than nexine (LM), exine ornamentation psilate (LM), fossulate-granulate (SEM) as shown in (Table 8, Plate 47).

Type II

Pollen, monad, outline circular, polar axis 14–18 µm (LM), 15–16 µm (SEM), equatorial diameter 14–17 µm (LM), 13–14 µm (SEM), exine thickness 1.2–1.7 µm (LM), sexine thinner than nexine (LM), ornamentation psilate (LM), fossulate-granulate (SEM) as shown in (Table 8, Plate 47).

Type III

Pollen, monad, outline circular, polar axis 14–19 µm (LM), 17–18 µm (SEM), equatorial diameter 13–16 µm (LM), 12–13 µm (SEM), exine thickness 1–1.2 µm (LM), sexine thinner than nexine (LM), ornamentation psilate (LM), fossulate-granulate (SEM) as shown in (Table 8, Plate 47).

Remarks: Palynological records were investigated from the Cenozoic of Eurasia and America. In south China sea *Moraceoipollenites* and *Engelhardtioidites* have been investigated, which resemble the reported Moraceae pollen grains (Zhi-Chen, 2004).

i) Myricaceae

Type I

Pollen, monad, outline triangular in polar view, exine thickness 0.6–0.8 µm (LM), equatorial diameter 16–17 µm (LM), 19–20 µm (SEM), triporate; ornamentation psilate-scabrate (LM), perforate, nano-echinate (SEM) as shown in (Table 8, Plate 48).

Type II

Pollen, monad, outline triangular in polar view, exine thickness 0.7–1 μm (LM), equatorial diameter 18-19 μm (LM), 15-16 μm (SEM), triporate; ornamentation psilate-scabrate (LM), perforate, nano-echinate (SEM) as shown in (Table 8, Plate 48).

Type III

Pollen, monad, outline triangular in polar view, equatorial diameter 21–22 μm (LM), 17–18 μm (SEM), exine thickness 1.6–2.4 μm (LM), triporate; ornamentation psilate-scabrate (LM), perforate, nanoechinate (SEM) shown in (Table 8, Plate 48).

Remarks: Myricaceae pollen grains is difficult to distinguish due to its similar morphological features (Punt and Marks 2003). It is anemophilous in nature and exists in swamps, lowland, and riparian forests.

j) Rutaceae

Rutaceae Type I

Pollen, monad, outline elliptical in equatorial view, equatorial diameter 13–14 μm (LM), 16–17 μm (SEM), polar axis 18–19 μm (LM), 21–22 μm (SEM), exine thickness 0.7–0.1.1 μm (LM), tricolporate, exine ornamentation reticulate (LM and SEM), striated (SEM) as shown in (Table 8, Plate 49).

Rutaceae Type II

Pollen, monad, outline elliptical in equatorial view, equatorial diameter 14–15 μm (LM), 17–18 μm (SEM), polar axis 19–20 μm (LM), 20–21 μm (SEM), exine thickness 0.9–1.2 μm (LM), tricolporate, exine ornamentation reticulate (LM), striato-reticulate (SEM) as shown in (Table 8, Plate 49).

Remarks: Members of the Rutaceae are mostly entomophilous and thrive in upland and lowland forests. Cao *et al*., 2014) reported the *Zanthoxylum*, which resembles many extant fossils.

k) Sapindaceae

Pollen, monad, outline circular in polar view, exine thickness 0.7–0.9 µm (LM), equatorial diameter 24–26 µm (LM), 13–15 µm (SEM), sexine and nexine thickness equal (SEM), colpi elongated, tricolpate, exine ornamentation striate (LM & SEM) as shown in (Table 8, Plate 49).

Remarks: Wood fragments of the remaining plant body have been reported from different regions of India in the Paleocene (Singh *et al*., 2011). Based on the morphological features, it is striato-reticulate (eFlora, 2008).

3.3.4. Spores

a) Monolete spore

Spore, monad, outline circular-elliptical, long equatorial diameter 40–41 µm (LM), 38–39 µm (SEM), short equatorial diameter 26–27 µm (LM), 24–25 µm (SEM), exospore thickness 0.6–1 µm (LM), sexine thinner than nexine, ornamentation psilate (LM & SEM) as shown in (Table 8, Plate 50).

Remarks: These kinds of spores were morphologically reported within Vittariaceae, Polypodiaceae, and Aspleniaceae. The specimen has monolete mark and exospore psilate exine ornamentation examined in the Cenozoic era of Europe (Stuchlik, 2001).

b) Trilete spore

Spore, monad, outline lobed in polar view, exine thickness 1-1.7 µm (LM), equatorial diameter 28-38 µm (LM), 25-33 µm (SEM), sexine thinner than nexine (SEM), ornamentation psilate (LM, SEM), laesurae perforated (SEM) as shown in (Table 8, Plate 50).

Remarks: Palynological record of the fossil pollen showed that the ridges of pollen grains have equatorial cingulum, large grana and small veruccae on the proximal surface, which resemble this spore (Korasidis *et al*., 2023).

c) Schizaeaceae

Spore, short equatorial diameter 29–30 µm (LM), 25–26 µm (SEM), long equatorial diameter 40–41 µm (LM), 37–38 µm (SEM), exospore thickness 0.8–1.2 µm (LM), Plicate (SEM) as shown in (Table 8, Plate 50).

Remarks: Pollen of Schizaeaceae have irregular and striate exine ornamentation reported from the Cenozoic of Eurasia (Avramenko & Polevova 2013).

3.3.5. Discussion

In this study, a total of 42 pollen grains belonging to angiosperm monocots, dicots, gymnosperms, and spores were identified. Styraceae is considered to be the dominant family, having 5 species, followed by Ericaceae (03), Fagaceae (03), Nyssaceae (02), Juglandaceae (02), Plantaceae (02), Santalaceae (02) Chloranthaceae (01), Typhaceae (01), Altingiaceae (liquidombor-01), Buxaceae (01), Caprifoliaceae (Viburnum-01), Eucommiaceae (Eucommia-01), Euphorbiaceae (01), Hammelidaceae (01), Loranthaceae (01), Malvaceae (01), Myricaceae (01), Oleaceae (01), Rosaceae (01), Rutaceae (01), sympolaceae (01), Thymelaceae (01), Ulmaceae (01), Ephedraceae (01), Pinaceae (01) and trilete spore (01). Using the single grain method, the palynomorphs were examined with microscopic techniques. With the help of previously published literature, the palynomorphs were identified up to the genus and family level.

The comparison of LM & SEM showed the present investigated species of Styraceae resemble its extant and fossil species reported from Europe (Hofmann *et al*., 2022). It is widely spread in the tropical and warm temperate areas of America, Europe, and Asia. In the current study, the pollen grains investigated were triangular, tricolporte, P/E rato prolate, and sculpture pailate-aerolate, while in the previous study they were tricolporate, prolate, and tectum regulate-fossulate. In this study, Fagaceae (*Quercus*) pollen grains were observed to be tricolporate, fosulate-regulate, and perforate, while in the previous study they were seen to be tricorporate, prolate, perforate, regulated, and micro-verrucate ornamentations. The pollen grains of current analyzed *Quercus* and Castaneoideae showed much similarity to the previous study. In the Eocene flora of Primorskii krai, megafossils of *Quercus, Castanea, Fagus, Lithocacarpus* and *Castanopsis* have been reported from the Eocene, showing high diversity of Fgaceae (Naryshkina & Evstigneev 2020; Hofmann, 2010). Pollen grains of the Ericacace observed in this study were quadrangular, sculpture irregularly scabrate, P/E ratio isodiametric, and granulate sculpture. The pollen grains studied in the previous study were tetrads collected from China, America, Germany, and Austria. Exine sculpture was regulated, and visin threads surrounded the pollen grain completely, which looked to be granular, not completely smooth, and exine sculpture was regulated (Zetter & Hesse 1996).

Pollen grains of Oleaceae observed in this study were tricorporate, sculpture reticulate, nano-echinate suprasculpture, while in previous studies it has been recorded to be tricolporate with small pores, microechinate suprasculpture, and reticulate sculpture (Kmenta & Zetter, 2013). The palynological record of *Olea, Phillyrea* and *Fraxinus* from the Cenozoic sediments of Altmittweida, Saxony and Germany have been reported (Manchester *et al*., 2015). In current research work the Loranthaceae pollen grains were seen to be tricolporated, triangular, psilated in LM, and microbaculated (SEM). Fossil pollen of Loranthaceae was observed to be triangular with concave sides, syncolpate, and having distinctive differentiation in exine sculpture and aperture configuration. The LM and SEM studies showed that it was abundant in Greenland and central Europe in the middle Eocene (Manchester *et al*., 2015). Pollen grains of Typhaceae examined in this study were porated, reticulate sculpture, and miroreticulated-reticulated sculpture while the pollen grains of Typhaceae observed in the previous study were ulcerated, reticulate sculptures with reticulum crested by nanoechini. The fossil record of the Typhaceae (*Sporangium*) was observed in China, America, New Zealand, and Australia (Punt & Clarke, 1976). Altingiaceae pollen grains examined in this study were pantaporated, reticulated, pori elliptical, while in previous studies, *Semiliquidambar, Noronha, Liquidambar* and *Altingia* were perforated, pantaporated, and nano-echinated (Ickert-Bond & 2013). Thymelaceae pollen seen in this study was monad and had psilate-echinate sculpture, while in the previous study it was examined to have a crotonoid pattern of exine, and based on its architecture, four types of pollen grains were examined using LM, SEM, and TEM (Herber, 2002). Pollen grains of Rutaceae were seen to be tricolporate, striato-reticulate, and colpus membrane granulated, which resembles the previous results of Rutaceae (Tricolporopollenites) and have been reported from the lower Eocene of northwestern Tethyan region (Hofmann, 2011). Plantaceae pollen grains observed in this study were tricolpated, circular-elliptical, trilobate and reticulate sculpture, while the pollen grains examined in the previous study were trilobated, spheroidal or oblated, angular aperture, and tricolpated, colpi long, reaching polar area; lumina greater in mesocolpia, becoming smaller at colpi margins; sexine thick, reticulate, and quietly similar to the previous pollen (Korasidis *et al*., 2023).

Rosaceae pollen was seen to be tricolporated, prolate, and psilate-striate sculpture in this study, while pollen examined in the previous study was monad, tricolporate, endopori smaller than the width of colpi; colpi long, endopori circular, and thickened margins of endopori (Grímsson *et al*. 2016). Myricaceae pollen observed in present study was triangular, circular pore, funnel-shaped, and psilate-scabrate sculpture, while pollen grains observed in the previous study were seen to have triangular, triporate, and irregularly arranged micro-verrucae sculpture. Using different microscopic techniques, morphological features of *Morella* and *Myrica* were studied, which resemble our results (Punt *et al*., 1976). Pollen grains of the *Salix* in Salicaceae showed tricolpate, reticulate-aerolate columellae, while pollen grains of the fossil Salicaceae showed Tricolporoidate, outline circular-elliptical in equatorial view, and exine sculpture microreticulate-verrucaed (Hofmann, 2018). Pollen grains of the Juglandaceae were seen to be triangular in polar view, elliptical in equatorial view, and sculptured psilate-granulate, while the pollen grains of the fossil Juglandaceae were examined to be spheroidal-oblate, heteropolar, and micro-gemmae-echinate. Nowadays, the genus *Viburnum* consists primarily of deciduous temperate woody plants from the northern hemisphere and a few evergreen plants from mountainous South America and SE Asia (Winkworth and Donoghue, 2005). The pollen grain of the *llex* was distinctive, and it has been found in the Cainozoic sedimentary record all across the world (Muller 1981). *Craigia*-type pollen (Malvaceae) in this study resembles the morphological characteristics of *Craigia hainanensis from the* Changchang Formation. It is commonly spread from the Eocene to the Pliocene within the northern hemisphere (Jin *et al*., 2009). Pollen grains of the Sympolaceae were seen to be tricolporate, triangular, and having scabre-verrucate sculpture, while pollen grains seen in the prevuous study were tricolporate, triangular to trilobare, and slightly truncated apices in polar view, ambs are slightly concave in compressed form (Hofmann, 2019). Some of the swamp inhabitants, such as *Myrica*, *Nyssa*-type and Salicaceae-type pollen, showed a wet environment with periodically flowing or standing water. Some of the results and botanical affiliations of our pollen taxa shown here are either novel or new for the Eocene era of Europe, and many were not previously discussed systematically.

Table 8. Quantitative features of fossil plants pollen from Paleocene, Patala Formation, Pakistan

S. No	Species Name	Equatorial diameter (µm) LM	Equatorial diameter (µm) SEM	Polar axis (µm) LM	Polar axis (µm) SEM	Exine thickness (µm) LM
1.	Araceae	30–31	29–30	39-40	35–36	4.2–5.3
2.	Arecaceae Type I (Palm)	17–18	15–16	31–32	28–29	1.1–1.4
3.	Arecaceae Type II (Palm)	18–19	19–20	34–35	33–34	1.2–1.5
4.	Arecaceae Type III (Palm)	20–21	17–18	36–37	32–33	1.3–1.6
5.	Arecaceae Type IV (Palm)	19–20	17–18	35–36	31–32	1.1–1.4
6.	Arecaceae Type V (Palm)	17–18	16–17	43–44	40-41	1.3–1.5
7.	Arecaceae Type VI (Palm)	15–16	16-17	37–38	36–37	1.6–2.1
8.	Arecaceae Type VII (Palm)	23–24	20–-21	33–34	31–32	1.1–1.4
9.	Arecaceae Type VIII (Palm)	15–16	11–12	27–28	22–23	1.4–1.8
10.	Arecaceae Type IX (Palm)	22–23	19–20	33–34	30–31	1.1–1.5
11.	Poaceae	31–32	29–30	47–48	33–34	2.4–2.9
12.	Cycadeaceae Type I	15–16	12–13	27–28	19–20	1.3–1.6
13.	Cycadeaceae Type II	17–18	16–17	35–36	30–31	2.1–2.5
14.	Rosaceae	23–24	18–19	33–34	32–33	1.7–2
15.	Sapotaceae Type I	28–29	26–27	23–24	21–22	2.3–2.9
16.	Sapotaceae Type II	19–20	22–23	21–22	24–25	1.2–1.5
17.	Anacardiaceae Type I	14–15	12–13	20–21	18–19	0.7–0.9
18.	Anacardiaceae Type II	21–22	13–14	28–29	16–17	0.9-1.2
19.	Cannabaceae	17–18	15–16	19–20	18–19	1.0–1.4
20.	*Diospyros* Type I	17–18	31–32	32–33	18–19	0.9–1.7
21.	*Diospyros* Type II	18–19	15–16	19–20	17–18	1.1–2.4

22.	Euphorbiaceae Type I	17–20	22–24	25–30	24-28	1.1–2.4
23.	Euphorbiaceae Type II	21–22	17–18	27–31	25–26	1.3–1.8
24.	Euphorbiaceae Type III	17–20	22–24	–	–	1.3–2.2
25.	Euphorbiaceae Type IV	18–19	20–21	26–29	23–25	1.5–2.7
26.	Fabaceae Type I	25–26	24–25	27–28	25–26	0.9–1.3
27.	Fabaceae Type II	21–22	18–19	24–25	21–22	0.7–1
28.	Moraceae Type I	13–18	14–15	13–16	12–13	0.7–0.9
29.	Moraceae Type II	14–18	15–16	14–17	13–14	1.2–1.7
30.	Moraceae Type III	13–16	12–13	14–19	17–18	1–1.2
31.	Myricaceae Type I	16–17	19–20	–	–	0.6–0.8
32.	Myricaceae Type II	18–19	18-19	–	–	0.7–1
33.	Myricaceae Type III	21–22	17–18	–	–	1.6–2.4
34.	Rutaceae Type I	13–14	16–17	18–19	21–22	0.7–1.1
35.	Rutaceae Type II	14-15	17–18	19–20	20–21	0.9–1.2
36.	Sapindaceae	24-26	13–15	–	–	0.7–0.9
37.	Monolete spore	40–41	38–39	26–27	24–25	0.6–1
38.	Trilete spore	28–38	25–33	–	–	1–1.7
39.	Schizaeaceae	40–41	37–38	29–30	25–26	0.8–1.2

Table 9. Quantitative features of fossil plants pollen from Paleocene, Patala Formation, Pakistan.

S.No	Taxon	Pollen Outline in Equatorial View	Pollen Outline in Polar View	Number of Colpi/Sulci/Pori	Exine Sculpture (LM)	Exine Sculpture (SEM)
1.	Araceae	Elliptical	-	-	Psilate	Foveolate-microperforate
2.	Arecaceae Type I (Palm)	Elliptical	-	Monosulcate	Psilate	Foveolate-microperforate
3.	Arecaceae Type II (Palm)	Elliptical	-	Monosulcate	Psilate	Foveolate-microperforate
4.	Arecaceae Type III (Palm)	Elliptical	-	Monosulcate	Psilate	Foveolate-microperforate
5.	Arecaceae Type IV (Palm)	Elliptical	-	-	Psilate	Foveolate-microperforate
6.	Arecaceae Type V (Palm)	Elliptical	-	Monosulcate	Psilate	Foveolate-microperforate
7.	Arecaceae Type VI (Palm)	Elliptical	-	Monosulcate	Psilate	Foveolate-microperforate
8.	Arecaceae Type VII (Palm)	Elliptical	-	Monosulcate	Psilate	Foveolate-microperforate
9.	Arecaceae Type VIII (Palm)	Elliptical	-	Monosulcate	Psilate	Foveolate-microperforate
10.	Arecaceae Type IX (Palm)	Elliptical	-	-	Psilate	Foveolate-microperforate
11.	Poaceae	Elliptical	-	-	Scabrate	Fosulate-perforate
12.	Cycadeaceae Type I	Elliptical	-	-	Scabrate	Fosulate-perforate
13.	Cycadeaceae Type II	Elliptical	-	-	Scabrate	Fosulate-perforate
14.	Rosaceae	Elliptical	-	-	Striate	Perforate
15.	Sapotaceae Type I	Elliptical-circular	-	Tricolpate	Psilate-scabrate	Microrugulate-perforate
16.	Sapotaceae Type II	Elliptical-circular	-	Tricolpate	Psilate-scabrate	Microrugulate-perforate
17.	Anacardiaceae Type I	Elliptical	-	Tricolpate	Scabrate	Striate
18.	Anacardiaceae Type II	Elliptical	-	Tricolpate	Scabrate	Microreticulate-
19.	Cannabaceae	Circular	-	-	Psilate	Perforate-nanogemmate
20.	*Diospyros* Type I	Circular	-	-	Psilate	Regulate-perforate
21.	*Diospyros* Type II	Circular	Circular	-	Psilate	Regulate-perforate
22.	Euphorbiaceae Type I	Elliptical	-	Tricolpate	Psilate	Verrucate-grnulate

23.	Euphorbiaceae Type II	Elliptical	-	Tricolpate	Psilate	Verrucate-grnulate
24.	Euphorbiaceae Type III	Elliptical	-	Tricolpate	Psilate	Reticulate
25.	Euphorbiaceae Type IV	Elliptical	-	Tricolpate	Psilate	Reticulate
26.	Fabaceae Type I	Circular	-	-	Scabrate-reticulate	Reticulate
27.	Fabaceae Type II	Circular	-	-	Scabrate-reticulate	Reticulate
28.	Moraceae Type I	Circular	-	-	Psilate	fossulate-granulate
29.	Moraceae Type II	Circular	-	-	Psilate	fossulate-granulate
30.	Moraceae Type III	Circular	-	-	Psilate	fossulate-granulate
31.	Myricaceae Type I	-	Triangular	Triporate	Psilate-scabrate	Perforate and nanoechinate
32.	Myricaceae Type I	-	Triangular	Triporate	Psilate-scabrate	Perforate and nanoechinate
33.	Myricaceae Type III	-	Triangular	Triporate	Psilate-scabrate	Perforate and nanoechinate
34.	Rutaceae Type I	Elliptical	-	Tricolporate	Reticulate	Striato-reticulate
35.	Rutaceae Type II	Elliptical	-	Tricolporate	Reticulate	Striato-reticulate
36.	Sapindaceae	Circular	-	Tricolpate	Striate	Striate
37.	Monolete spore	Circular	-	-	Psilate	Psilate
38.	Trilete spore	-	Triangular	-	Psilate	Psilate
39.	Schizaeaceae	Circular	-	-	Plicate	Plicate

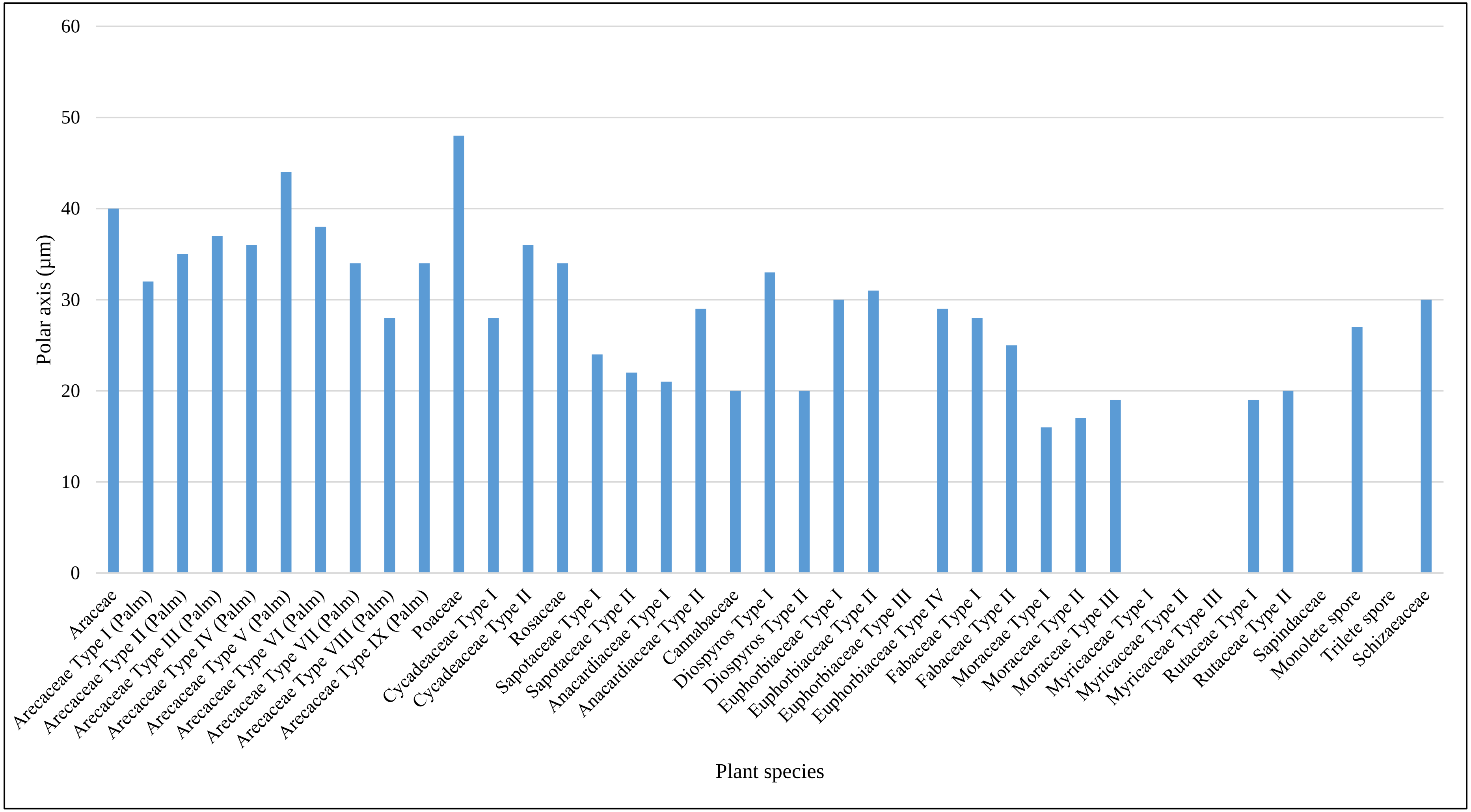

Figure 13. Variations in polar axis among pollen grains of Paleocene, Patala Formation, Pakistan observed through LM.

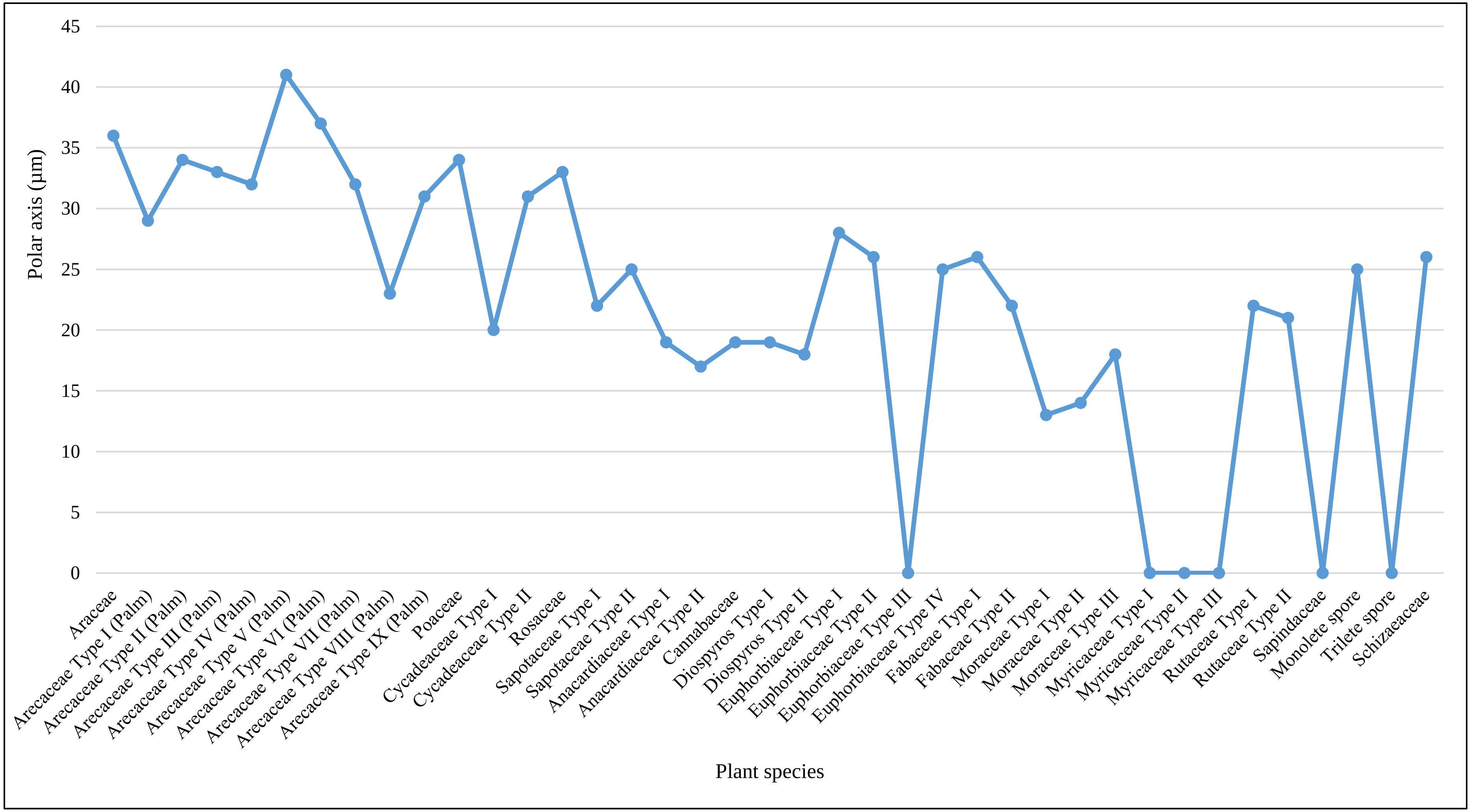

Figure 14. Variations in polar axis among pollen grains of Paleocene, Patala Formation, Pakistan observed through SEM.

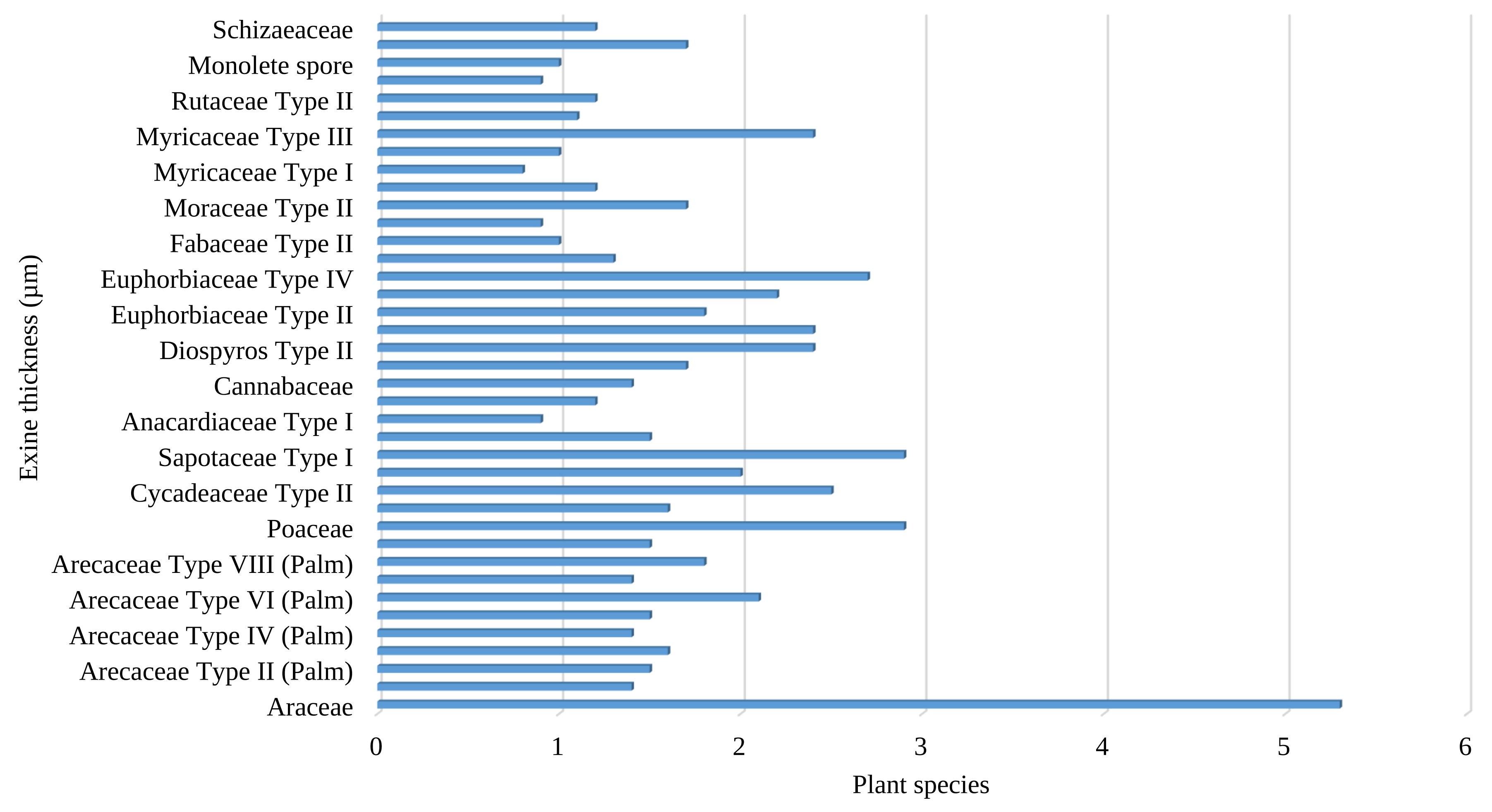

Figure 15. Variations in values of exine thickness in pollen grains of Paleocene, Patala Formation, Pakistan.

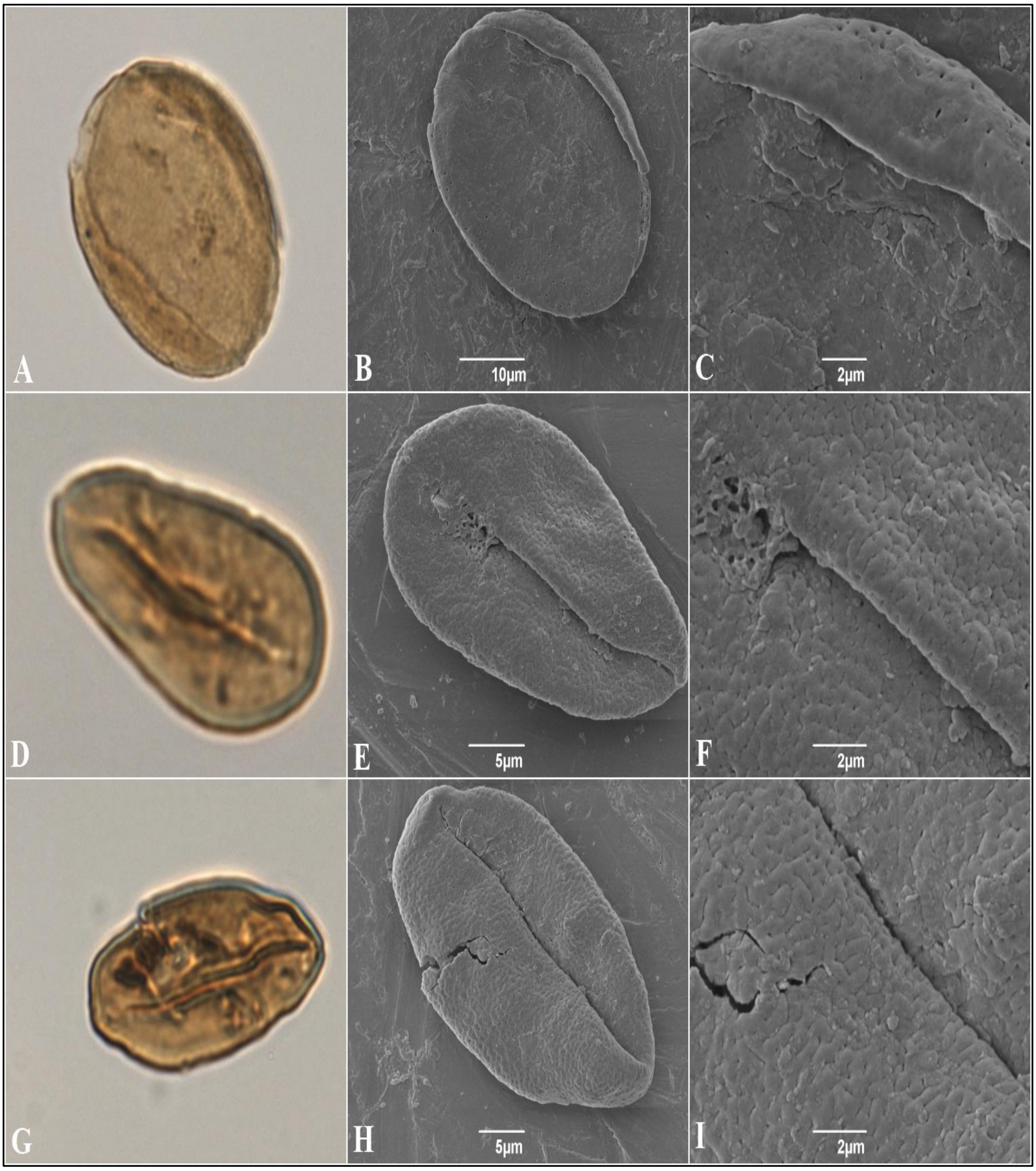

Plate 38. LM & SEM pollen photographs. A-C. Araceae; D-I. Arecaceae (Palm)

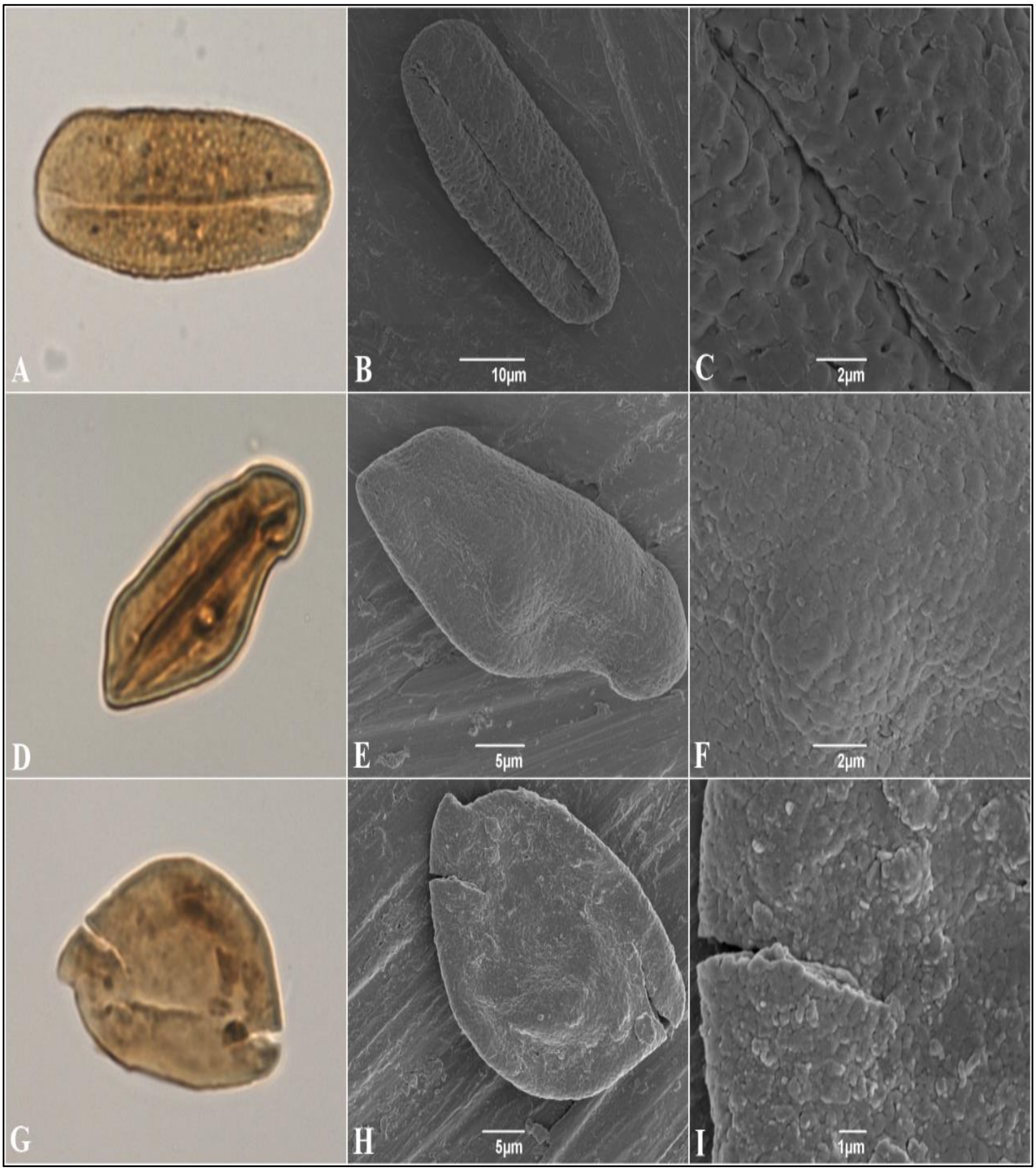

Plate 39. LM & SEM pollen photographs. A-I. Arecaceae **(**Palm**)**

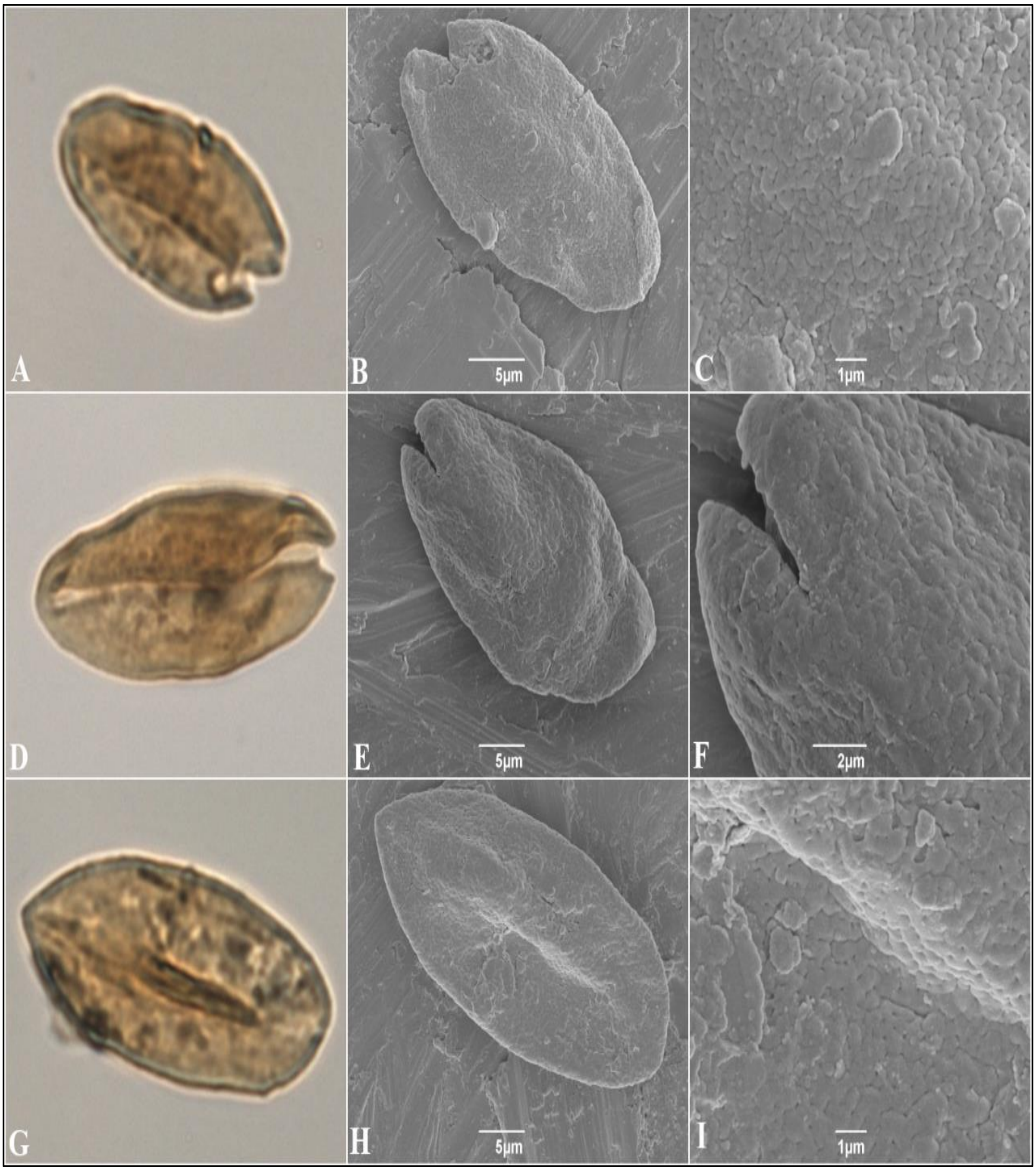

Plate 40. LM & SEM pollen photographs. A-I. Arecaceae **(**Palm)

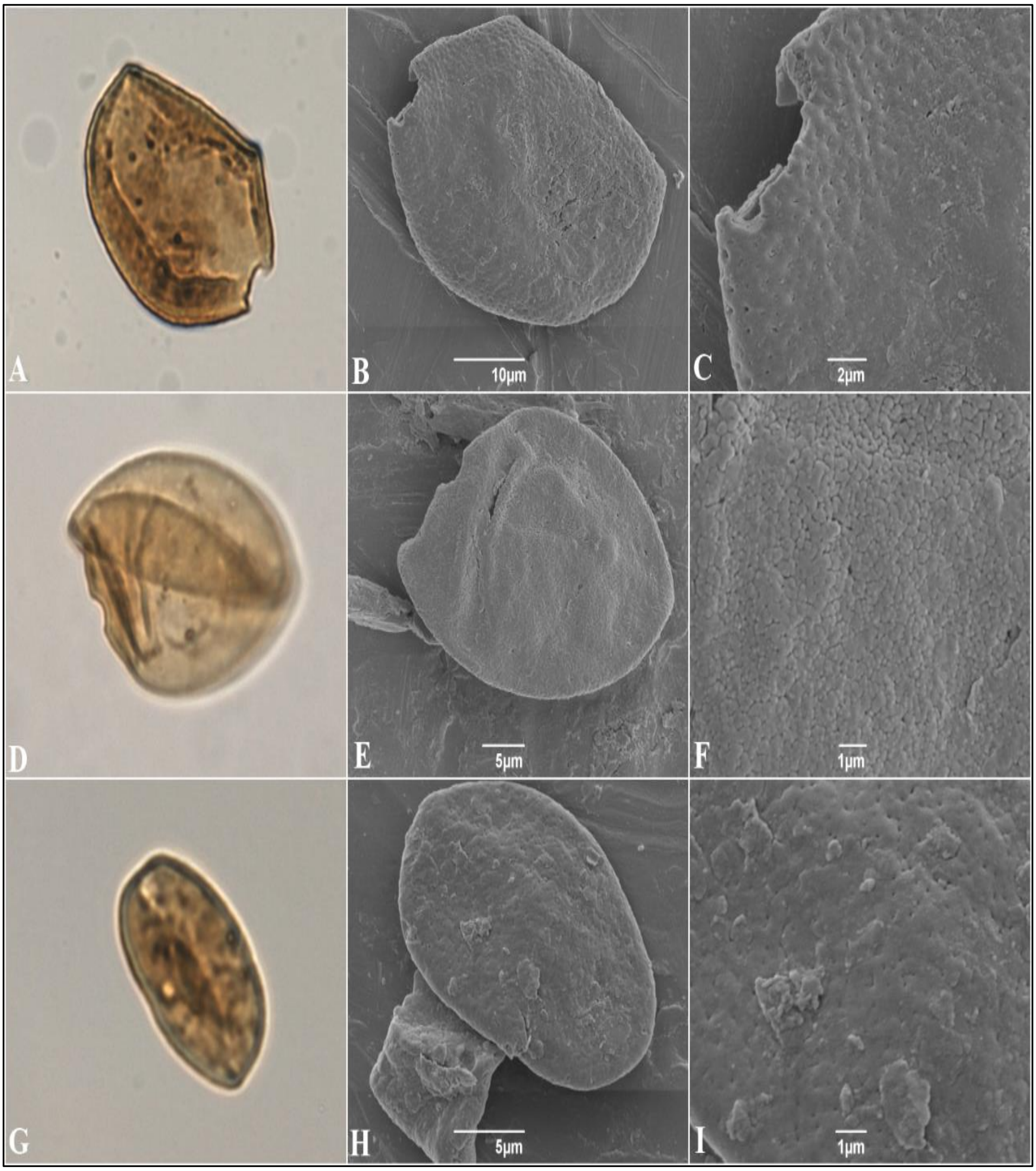

Plate 41. LM & SEM pollen photographs. A-C. Arecaceae Palm) **(**D-F. Poaceae; G-I. Cycadeaceae Type I

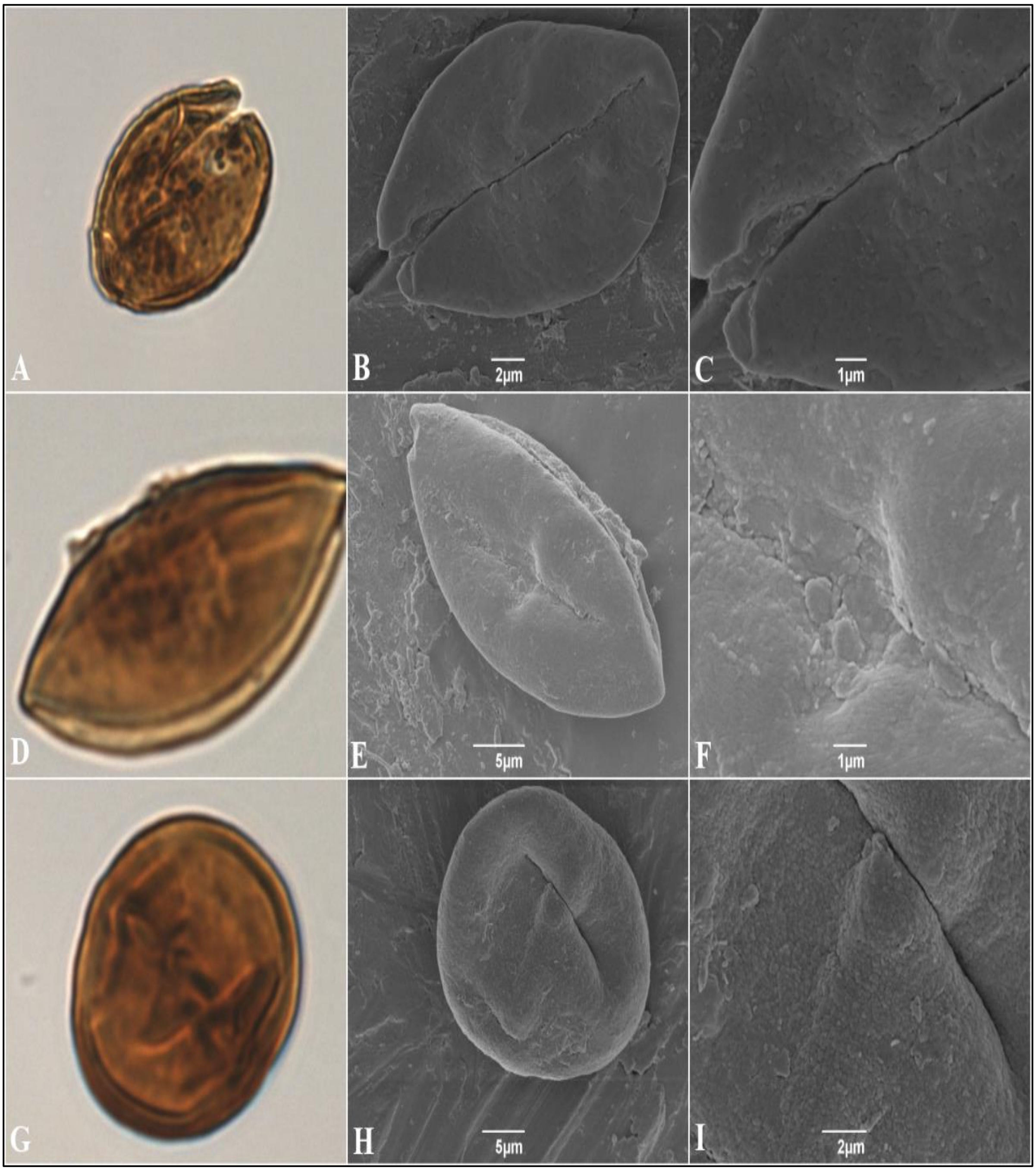

Plate 42. LM & SEM pollen photographs. A-C. Cycadeaceae Type II; D-F. Rosaceae; G-I. Sapotaceae Type I

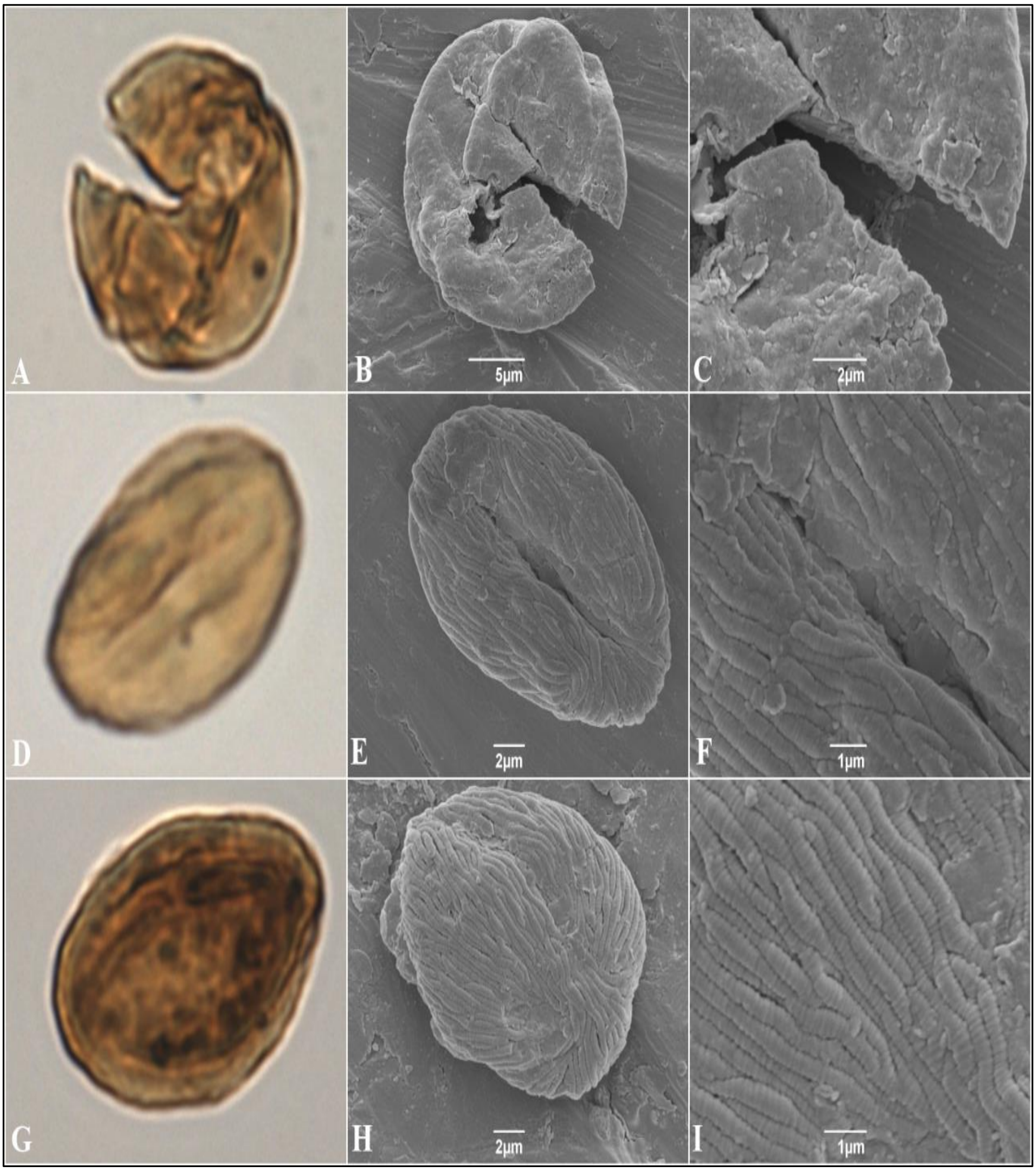

Plate 43. LM & SEM pollen photographs. A-C. Sapotaceae Type II; D-I. Anacardiaceae

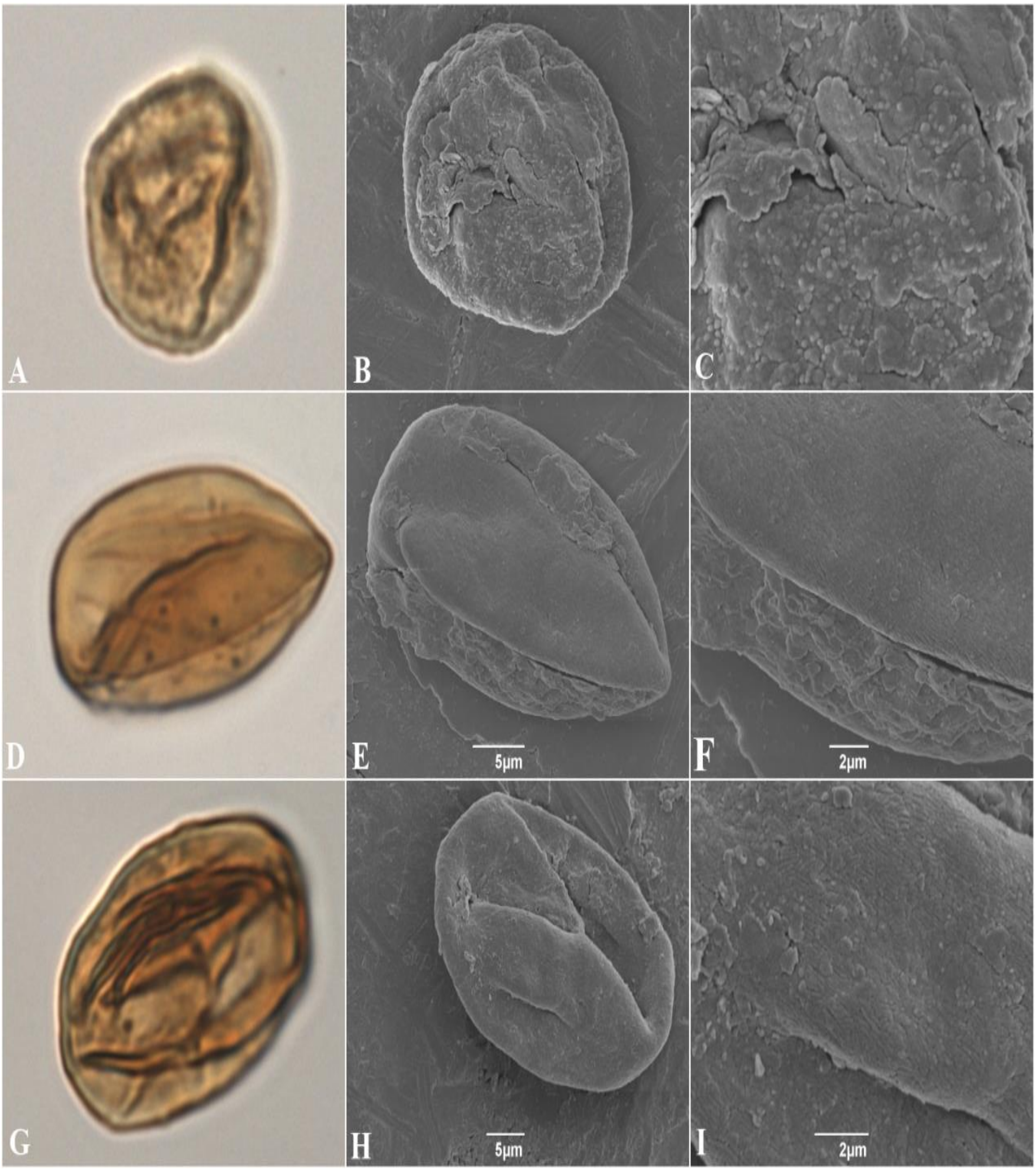

Plate 44. LM & SEM pollen photographs. A-C. Cannabaceae; D-I. *Diospyros*

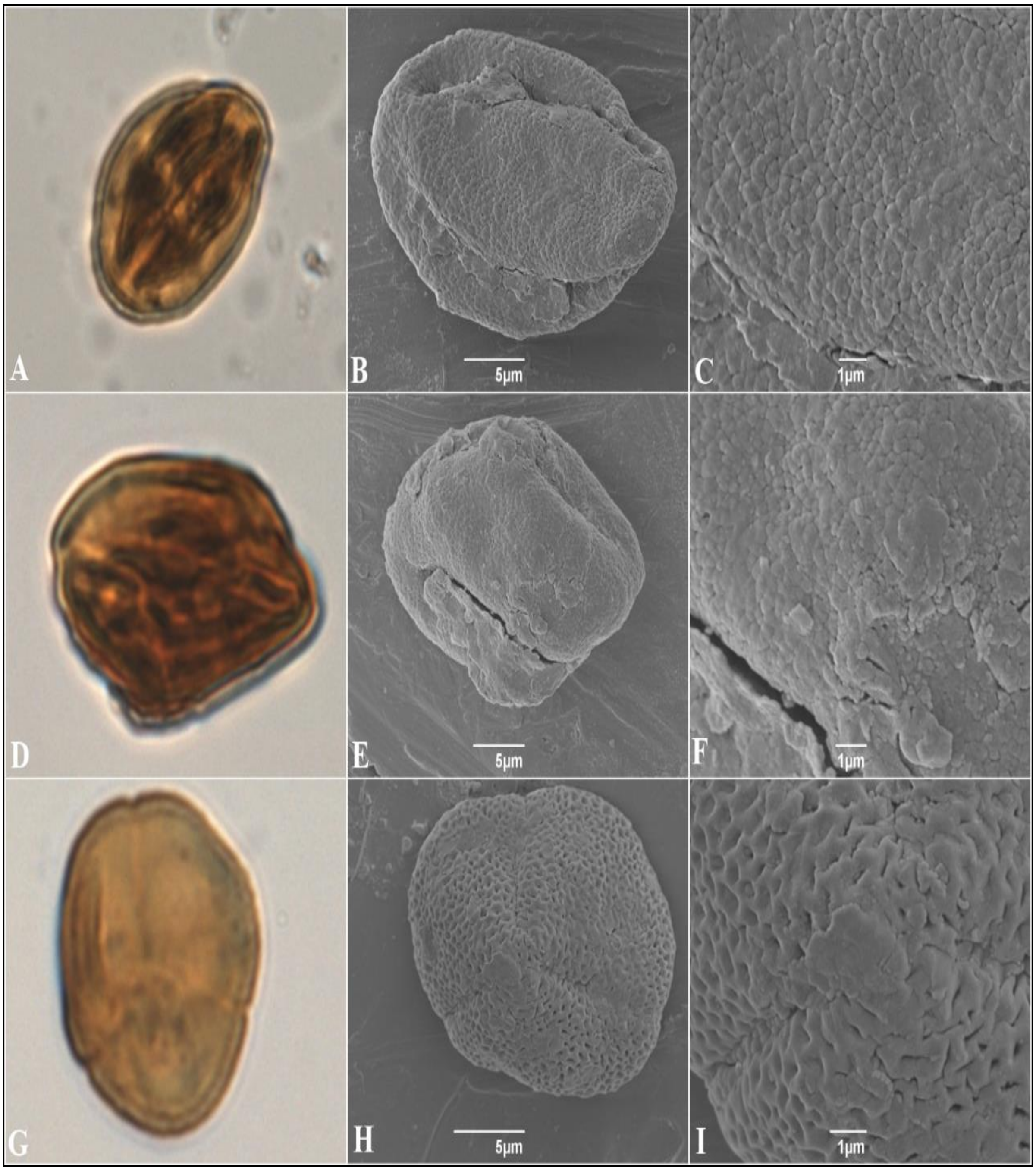

Plate 45. LM & SEM pollen photographs. A-I. Euphorbiaceae

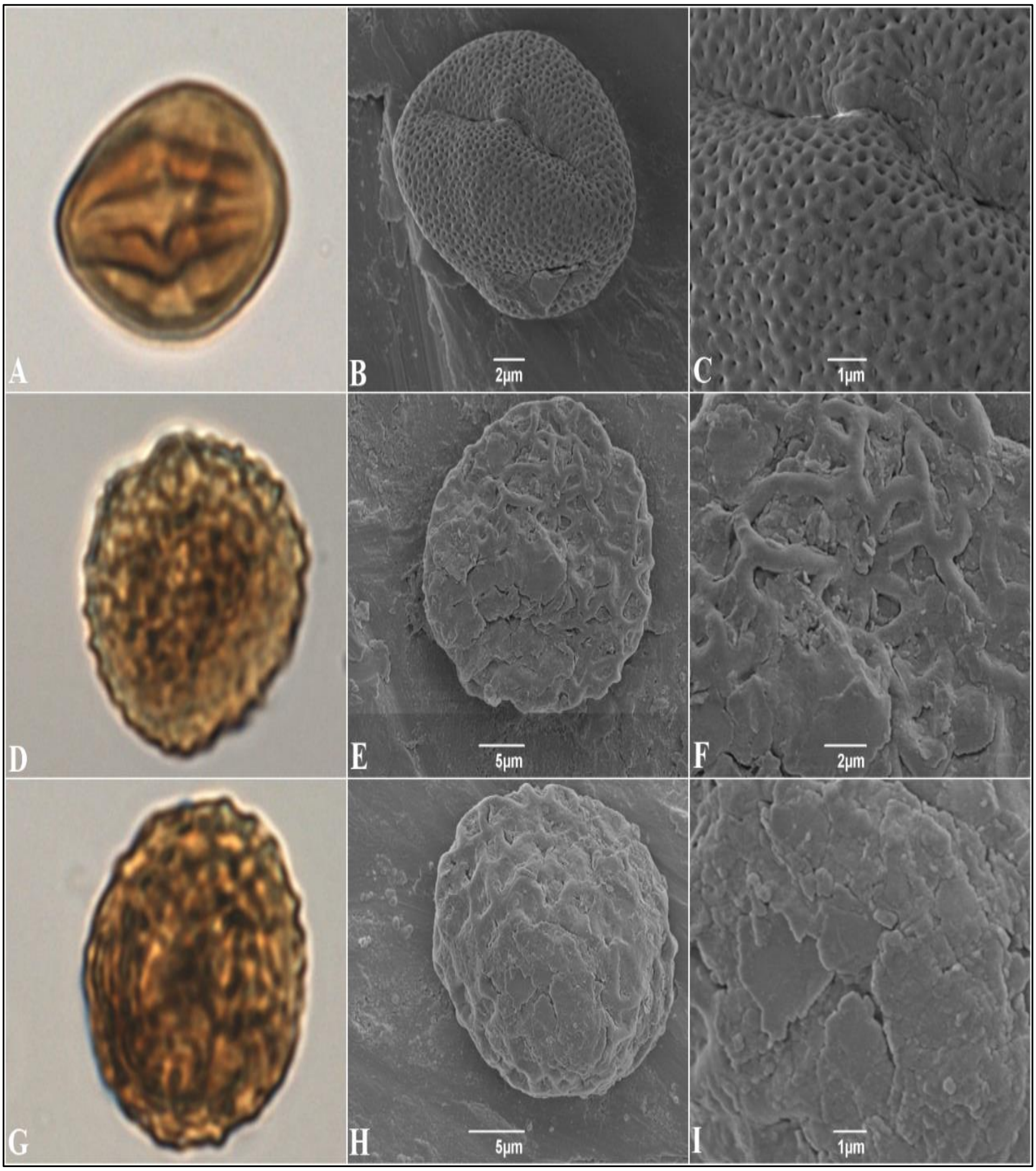

Plate 46. LM & SEM pollen photographs. A-C. Euphorbiaceae; D-I. Fabaceae

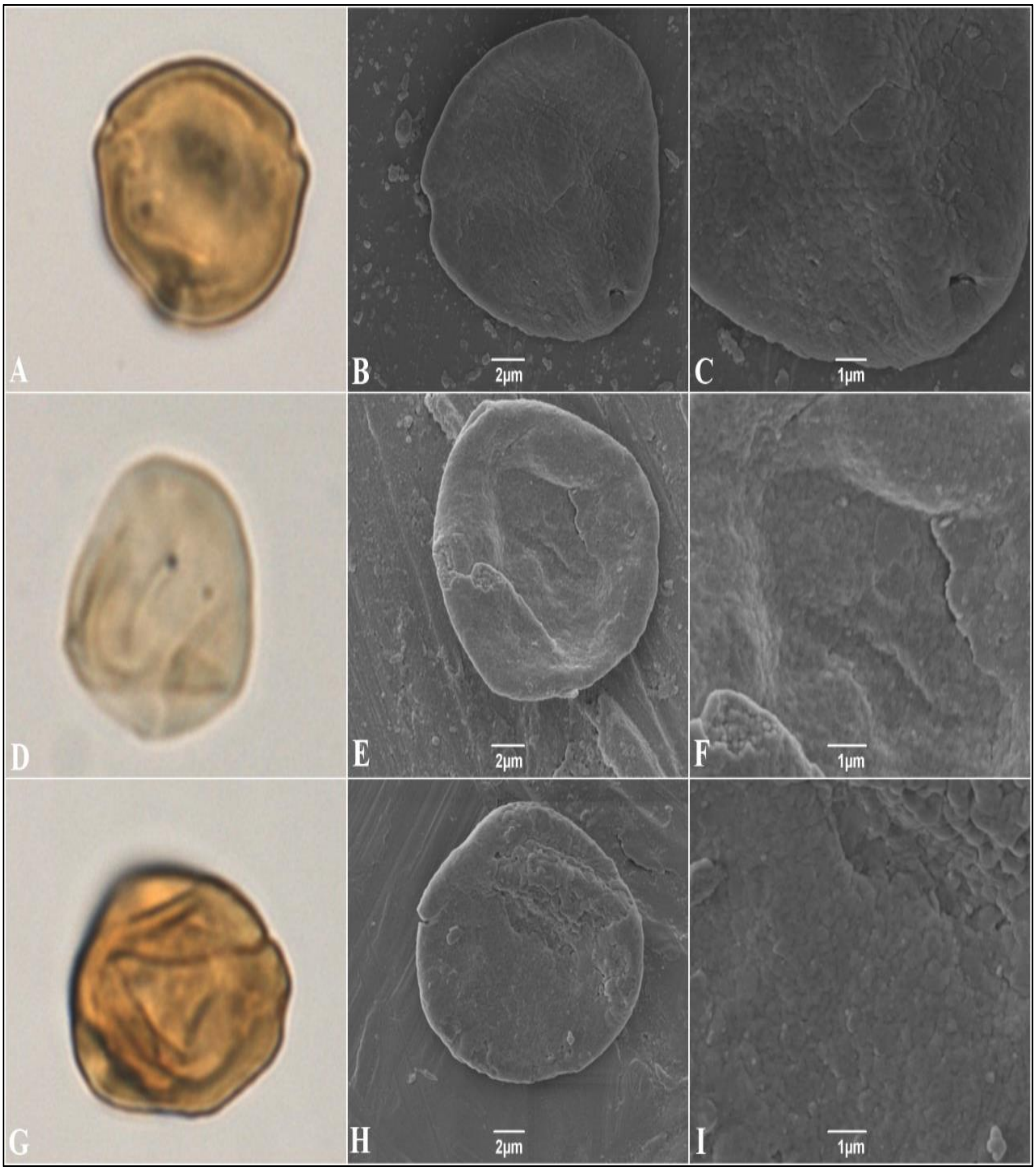

Plate 47. LM & SEM pollen photographs. A-I. Moraceae

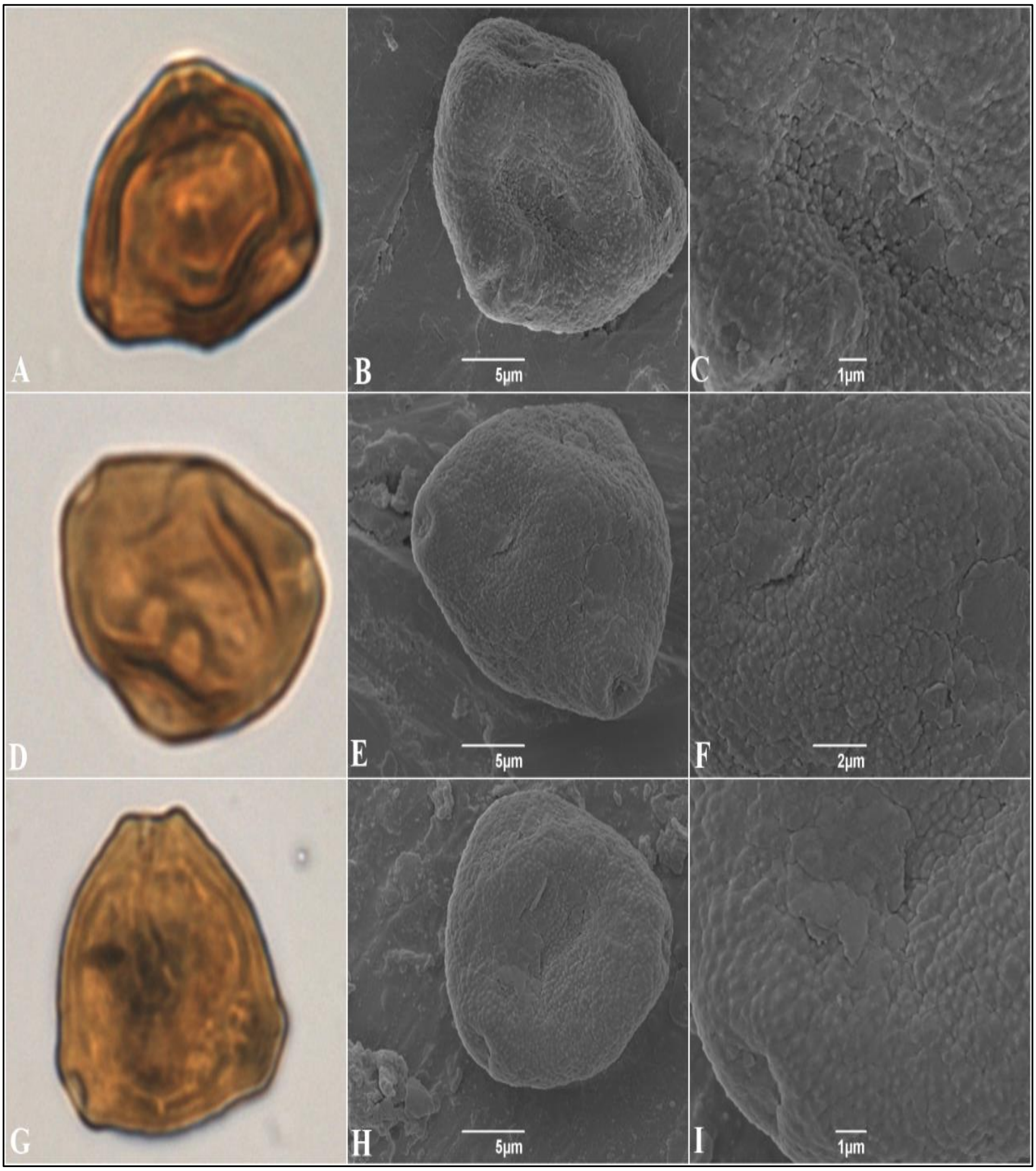

Plate 48. LM & SEM pollen photographs. A-I. Myricaceae

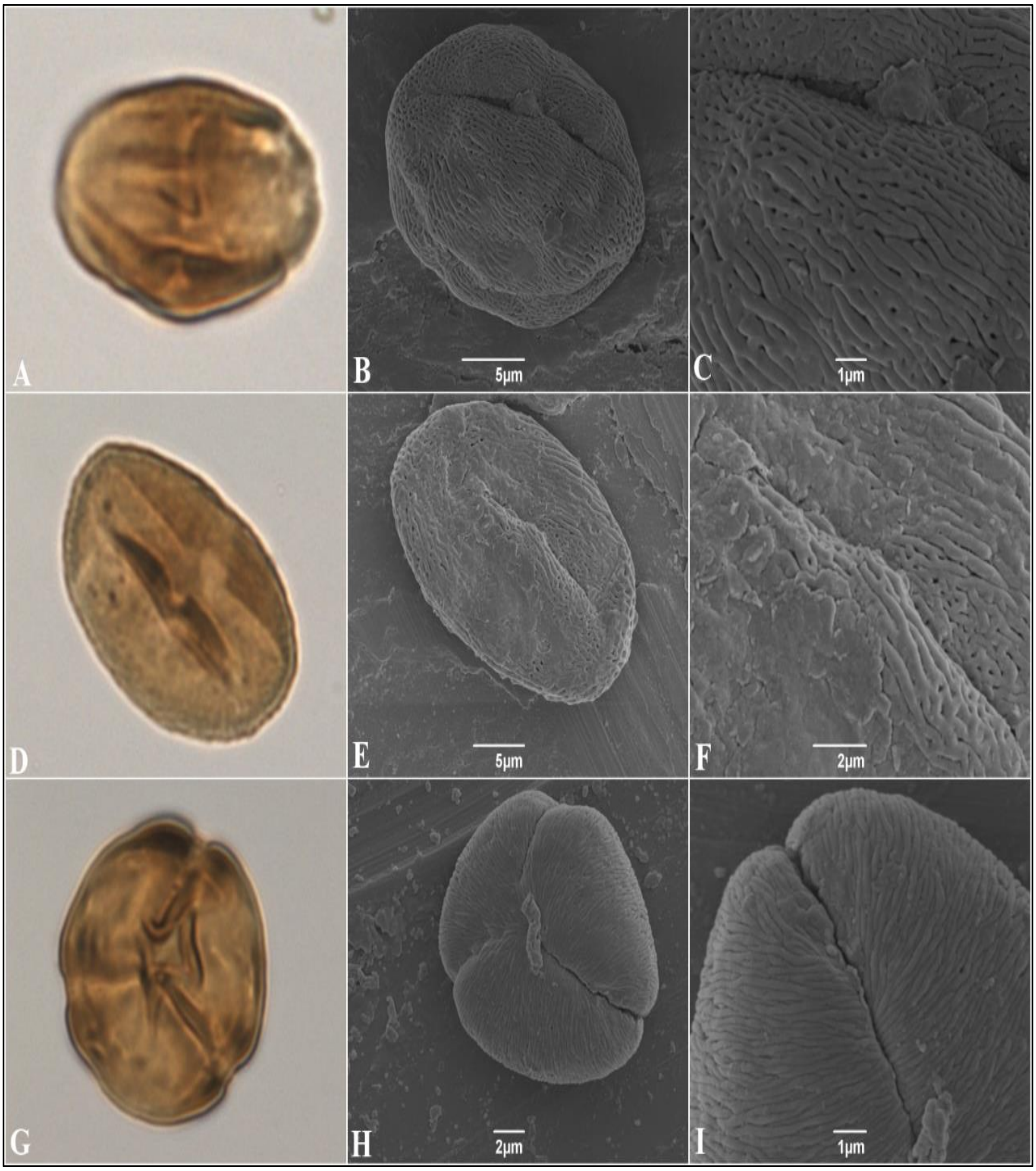

Plate 49. LM & SEM pollen photographs. A-F. Rutaceae; G-I. Sapindaceae

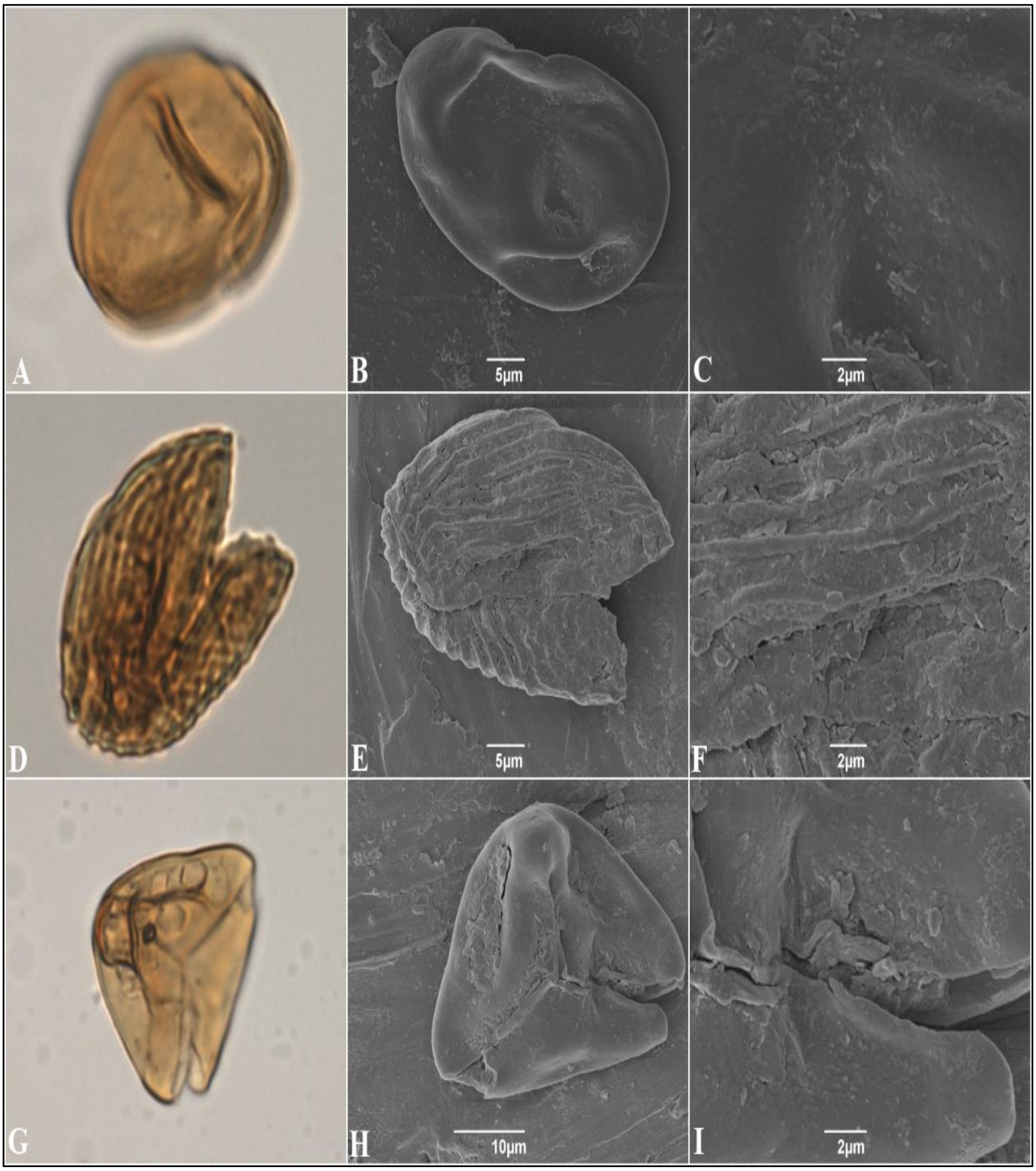

Plate 50. LM & SEM pollen photographs. A-C. Monolete spore; E-G. Shizaceae; H-I. Trilete spore

SECTION: II

Conclusion and Future Recommendations

Conclusion

In the present study, a total of 161 palynomorphs were identified and described up to genus and family level using microscopic techniques from the selected microflora of Cenozoic Eurasia, providing a detailed account of the climatic and vegetation variations within the study area. Based on the palynological analysis, it has been concluded that climatic fluctuations occurred within the study area and variations observed within the qualitative and quantitative characters of palynomorphs play a key role in the identification of species. With the help of the present study, the evolutionary record of past vegetation, its extinction events, diversifications, and reconstructions of climate will be determined. Using LM and SEM, a catalogue of palynomorphs was an urgent need with its taxonomic affinities, standardizing nomenclature, and documenting the palynological assemblages of fossil plants. Based on these data, we concluded that the Eocene-Miocene palynoflora shows similarity to the previous published literature within Eurasia. Further studies in the future are recommended to identify the vegetation at the taxon and genus level through advanced microscopic techniques like transmission electron microscopy (TEM). The Miocene palynoflora showed warm tropical genera along with temperate elements. Diverse and rich palynoflora from the coals of the Paleocene Patala formation indicate the presence of tropical rain forests. Angiosperm pollen grains are comparatively dominant as compared to spores and gymnosperms. The presence of some families like Fagaceae, Betulaceae, Juglandaceae, and Pinaceae shows the presence of subtropical-temperate vegetation within the base of mountainous areas. The fossil pollen record of Arecaceae, Malvaceae, and spores within the shallow marine environment in the Paleogene indicates the development of succession within the place influenced by brackish water. Modern vegetation has warm tropical genera along with temperate elements. Based on these data, it has been concluded that many taxa originated in the study area and are considered important to study the origin and development of lower and higher vascular plants.

Future Recommendations

The current research work in the field of paleopalynology is very useful in reconstructing the past vegetation and climate within the study area. The study needs to be compiled into a book with a pollen description and its LM and SEM photos to serve paleontologists, botanists, and geologists. Many of the palynomorphs are difficult to identify, and to solve the problem of identifying them correctly, we need to establish the pollen atlas, which requires a lot of funding. The study needs to identify the pollen up to the species level using TEM instead of LM and SEM. The study will help to compare the pollen reconstruction with earth model system and paleoenvironment proxies.

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QUAID-I-AZAM UNIVERSITY
DEPARTMENT OF PLANT SCIENCES

Subject: **Publication of W – Category Mr. Shabir Ahmad (Ph.D. Scholar)**

This is in reference to circular regarding the publication requirement for Ph.D. scholars in Department of Plant Sciences, Faculty of Biological Sciences. It is certified that **Mr. Shabir Ahmad** has published research papers in W-Categories as given below:

S. No.	Paper Title	Year	Impact Factor
1.	Ahmad, S., Ahmad, M., Fawzy Ramadan, M., Sultana, S., Papini, A., Ullah, F., Saqib, S., Ayaz, A., Ahmed Bazai, M., Zaman, W. and Zafar, M., 2023. Palynological Study of Fossil Plants from Miocene Murree Formation of Pakistan: Clues to Investigate Palaeoclimate and Palaeoenvironment. *Agronomy*, *13*(1), p.269.	2024	3.94

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Supervisor

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Article

Palynological Study of Fossil Plants from Miocene Murree Formation of Pakistan: Clues to Investigate Palaeoclimate and Palaeoenvironment

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Abstract: Palaeoflora in Pakistan in the Miocene is characterized by its high biodiversity. The present study investigated the pollen of fossil plants from the Murree Formation of Pakistan. Shales and mudstones were collected from the Murree section located at the foothills of the Margalla hills and analyzed by palynofacies and palynostratigraphic analyses. In this paleopalynological study of the Miocene Murree Formations of Pakistan, 31 samples were analyzed using microscopic techniques containing 48 pollen types from 12 families. The quantitative and qualitative morphological features of pollen were determined using light and scanning electron microscopy to help identify the pollen grains. Exine ornamentations and spines were the most important diagnostic features for distinguishing one pollen grain from another. The maximum exine thickness was observed in *Ocimum basilicum* of 4.25 µm, whereas the maximum pollen diameter was recorded for *Pinus* of 69.5 µm. Lamiaceae, Asteraceae, and Poaceae were the dominant families. The results showed that the preservation of floral records was not optimal. The presented results provide data on the dominant fossil plant taxa that existed in Pakistan (23.03–5.33 Ma). The evolution and phytogeographical histories of fossil plants can be unraveled using rock sediments to preserve biodiversity.

Keywords: palynoflora; taxonomic; microscopy; systematics; vegetation's origin

1. Introduction

In the tertiary period, the Himalayas formed as a result of a collision between the Eurasian and Indian plates [1]. The Himalayas have been further classified into higher Himalayas, lesser Himalayas, and sub-Himalayas and are considered the main source for the Murree Formation. This formation has a strong tectonic affinity regarding the structural evolution with the Himalaya orogeny. It has been named Murree owing to the locality of Murree hills in the district of Rawalpindi, Pakistan. The Rawalpindi formation lies in the early Miocene period [2]. Therefore, it provides important information on how the fossil plant pollen record in the core–mantle boundary relates to the Indo–Asian tectonic