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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 collision and the environmental change preceding the Miocene–Eocene Transition [3]. In Pakistan, the Murree Formation comprises the area of Islamabad, Kohat, Murree, and Attock districts. Regarding the current climate (temperature and precipitation), four seasons are recorded in the study area: summer, autumn, winter, and spring. The summer season is longer than others; it begins in April and ends in September. Geographically, the country comprises mountains, plateaus, rivers, deserts, and forests. The regions with lower altitudes exhibit tropical and subtropical climates, while higher altitudes possess temperate environments [4,5].

Pakistan has Gondwanan, Eurasian, and Tethyan ancestors of the vegetation components. The Indus basin of Pakistan, which is in the center, south, and east of the country, represents the Gondwanan heritage [6]. The sedimentary strata of the Murree Formation consist of purple, grey, and dark red sandstone formed from a monotonous sequence. The lower part consists of calcareous sandstone containing numerous foraminifera, generally known as Fatehjang. In the Attock district, one of the types of sections is exposed at the north of the Dhok Maiki (Lat. 33°25′ N: Long. 72°35′). The main body of the formation consists of fossiliferous, silicified wood, plant remains, and fish [7–9]. The current study analyzed some fossil plant pollen records from different families. Pollen and spores are essential sources to determine the climate variations of the study area. They are important investigative tools in several fields, including paleobotany, archaeology, and geography, regarding the climate and other environmental factors affecting flora in any area [10,11]. The Stratigraphic committee of Pakistan has worked on the stratigraphic nomenclature in the country [6]. Paleo-environmental changes in Asia occurred mainly in the Miocene period. Therefore, numerous relevant fossils of the period can be obtained from the corresponding stratum [12]. The sedimentary strata of the Murree Formation consist of various coarse to fine-grained units. In the Attock district, the Murree Formation comprises lamellated claystone, sandstone, and conglomerates. In Kohat, the formation is characterized by medium to coarse-grained sandstones [13]. In northern Potwar, the formation reaches its maximum thickness of approximately 3030 m, but in western Kohat, it thins out and is approximately 9 m thick [4]. The main deposition of the Murree Formation occurred in a fluviatile environment that was affected by meandering rivers and turbidity currents, as evidenced by cyclic deposition of shale, siltstones, and sandstones, as sedimentary structures, such as calcite concretions, ripple marks, cross-bedding, worm barrows, and lithofacies (Figure 1). The sediments of the Murree Formation contain fossil materials of plant and animal remains. For example, in Fathehjang, numerous mammal bones have been discovered, including remains from different even-toed ungulates, rhinoceros, and hippopotamus-like animals [7]. Based on the mammalian fossil record, the age of the Murree Formation has been constrained to the Miocene.

A study was conducted to estimate the effects of climate change on Pakistan's natural forest ecosystems, using 1990 as the base year and assuming a 0.3 °C rise in temperature and changes in precipitation of 0, +1, and −1% decade^{-1}. The study predicted that the atmospheric CO_2 concentration of 350 ppmv will rise to 500 ppmv in 2050 and 575 ppmv in 2080. The current climate of the country was reported to vary. Some major biomes were noted, such as croplands, mosaic vegetation, evergreen forest, mosaic grassland, open grassland, barren area, and permanent snow. Owing to various climatic conditions, the variations were investigated in different kinds of forests in the study area, i.e., alpine tundra, mixed woodland, cold conifer, warm conifer, xerophytic wood, grassland, steppe, and desert (Figure 1). Extreme biomes on either side of the spectrum are more vulnerable to climate change than others. The alpine tundra loses approximately 32% of its area to other biomes, particularly those directly beneath it (such as cold coniferous/mixed forest). On the other hand, warm conifers expand their range significantly when temperatures and precipitation rise. Climatic variations led to significant shifts in forest areas from one biome to another in northern Pakistan. The overall climate has a significant negative impact on the natural and forest ecosystem of Pakistan [13–15].

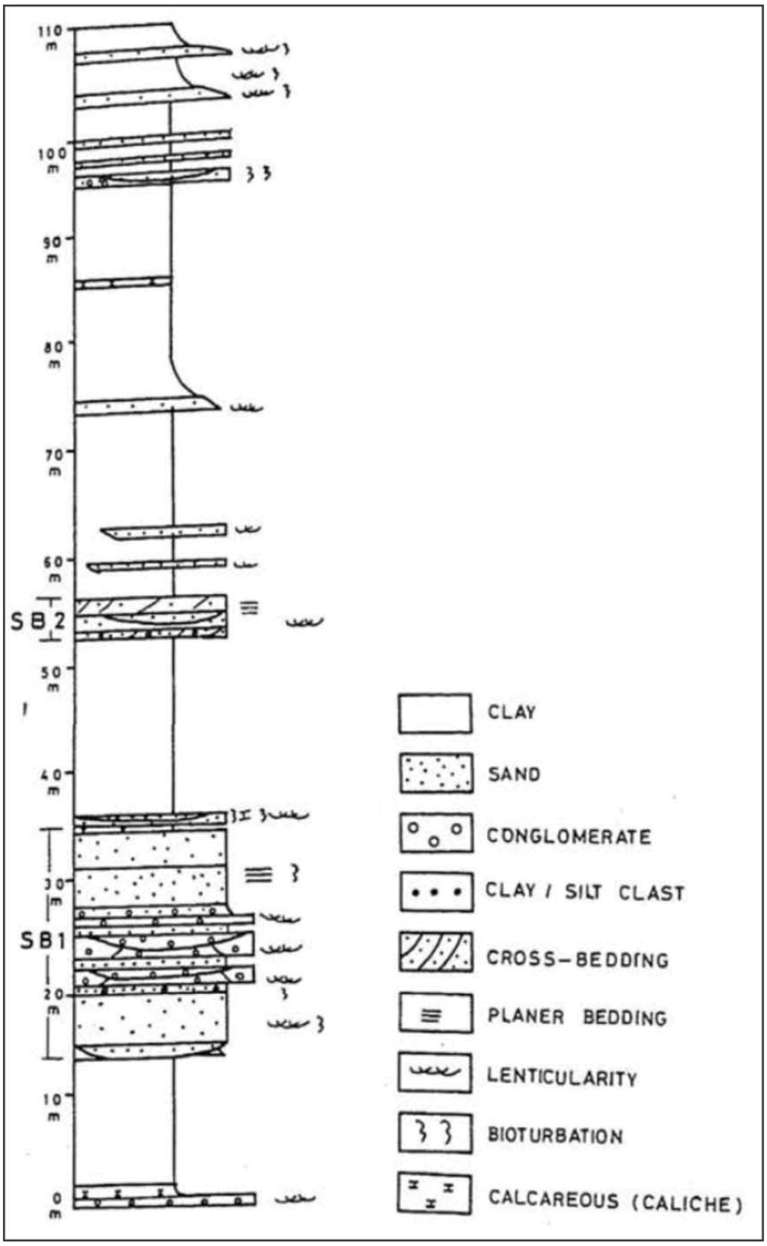

Figure 1. Stratigraphic column of the Murree Formation, Pakistan [13].

The multiple scenarios support the study area's rich and diverse vegetation. This diversity is reflected in the current flora of Pakistan (see http://legacy.tropicos.org/Project/Pakistan), where more than 6000 higher vascular plant species have been described, along with approximately 190 pteridophytes. Approximately 80% of the flowering plants were recorded. Important for this study is that the pollen morphology of plants occurring in various vegetation units of Pakistan is well documented. Optical and scanning electron microscopy-based pollen morphologies of over 70 plant families in Pakistan have already been published in a series of reputable papers [14]. These data make it practical to compare the fossil pollen grains to pollen from extant plants whose ancestors most likely occurred in the study area during the Miocene.

Palaeopalynology is the study of ancient spores and pollen occurring in sedimentary rocks. Pollen grains are the male reproductive parts (male gametophyte surrounded by a sporal wall) found in seed plants and show a very high degree of diversity in shapes and size [16,17]. The outer wall layer of the pollen grain is called the exine, while the inner layer is the intine; each is made up of several compounds, among which sporopollenin is the most important of the exine layer. Exine can be divided into a sexine (more external) and a more internal nexine. The wall of the pollen grain is resistant to the action of the external environmental conditions. In different families and genera, pollen varies in sculpturing, walls, size, shape diameters, colpi, and pores [18]. Spores are the long-distance dispersal units in seedless terrestrial plants, and monilophytes can also be used in paleopalynology. Pollen and the spore morphology also play a vital role in species identifications [19–21].

Palynological investigations in mountainous areas of Pakistan in the Miocene age are few and limited to some regions [22]. The primary goal of this investigation is to study the palynomorph assemblages found in the tidal and marine environments of the study area. The assemblages of palynoflora recovered from the rocks are relatively diverse and well-preserved. The floral characters were compared to those found in previous studies. In addition, several changes were made to the original matrix of morphological characters based on a previous study [23]. The following questions are addressed in the current study. What were the dominant types of vegetation in the investigated age? What were the biogeographic origins of Pakistan's modern flora? How did these pollen types compare with morphotaxa from Miocene records of adjacent regions?

The following approaches were made to answer all these questions. Palynomorphs' morphological features were investigated using qualitative and quantitative pollen characters to determine the paleo-climate of the past marine environment and further described along with the section near the Murree Formation. Paleopalynology knowledge is essential for knowing the past climate of the Miocene reconstruction of vegetation and paleo-environment. The purpose of this study was to investigate fossil plant pollen records preserved in the sedimentary strata of the Miocene and its correlation with the paleo-environment [12]. This research was very useful in investigating fossil pollen of the Miocene Murree Formation for the first time. The study helps reconstruct Miocene palaeovegetation and climatic changes occurring in the depositional environment. It also helps to estimate the regional palaeoclimate, paleophytogeographic history of the paleoflora and its relation to the modern flora of Pakistan.

2. Materials and Methods

The field surveys were conducted in November and December 2021 on the numerous different sequential horizons in the foothills of Margalla Islamabad, Pakistan, during which the latitude, longitude, rainfall, and temperature were recorded (Figure 2). The main deposition of the Murree Formation occurred in a fluviatile environment affected by meandering rivers and turbidity currents, as evidenced by the cyclic deposition of shales, siltstones, and sandstones, as well as sedimentary structures, such as calcite concretions, ripple marks, cross-bedding, worm barrows, and lithofacies.

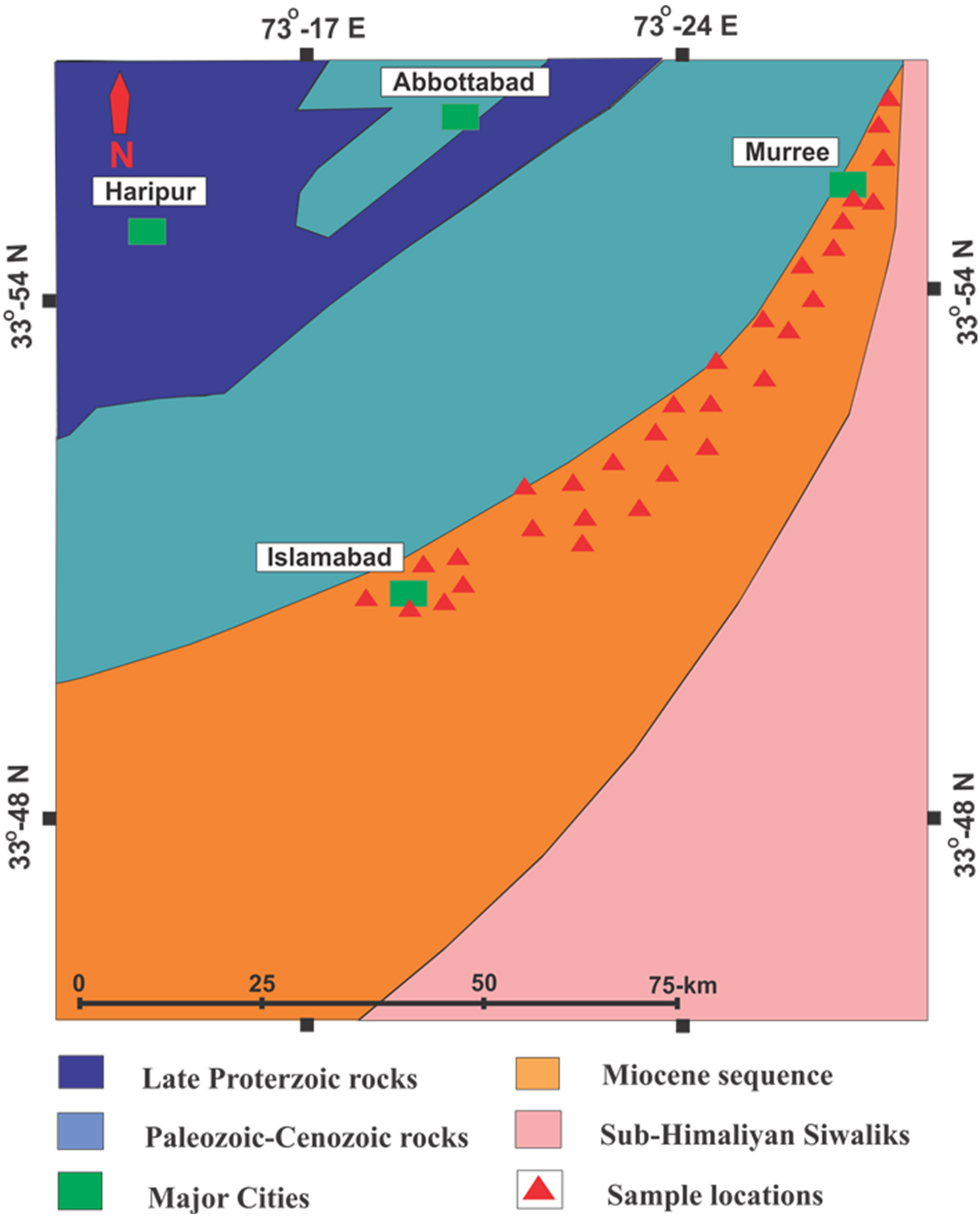

Figure 2. Map of the study area showing the sample localities [8].

2.1. Samples Collections

The Miocene Murree Formation has distinguished features, such as lithofacies, bed thickness, sedimentary structures, grain size, and color. Sandstones of the formation are generally fine-grained with a dominant reddish color followed by greenish and greyish color grains [13]. The study area is considered the Miocene Murree Formation based on these unique features and the discovery of the mammalian fossil record [7,13].

The samples were collected after digging 30 cm of sedimentary rock carefully to ensure that it contained palynomorphs (Figure 3). For paleopalynological purposes, 31 samples were collected from shales and mudstones along a 50 m thick Murree section and processed at the Plant Systematics and Biodiversity Laboratory, where all necessary instrumentations and chemicals are available. The samples were photographed, collected in polyethylene bags, and then brought to the laboratory for further processing. Palynological samples were also used to identify palynomorphs in plant taxonomy and systematics [24,25].

Figure 3. Field photography: (**A**) Hammer placed on the rock for cutting the rock sediments. (**B**) Sample collection from the study area. (**C**) Samples collected and placed in a polyethylene bag for palynological purposes. (**D**) View of the study area.

2.2. Sample Preparation

The preparation of sedimentary rock samples was undertaken using the standard protocol of palynology with some modifications [26,27]. The rock sediments were treated with 10% HCl for 1 day to remove carbonates and then treated with distilled water to neutralize them. The samples were again reacted with 40% HF for at least one day to remove silicate particles and treated with distilled water for neutralization. For further palynological processing, the samples were centrifuged at 2500 rpm to separate the heavy and light particles. Heavy liquid with a specific gravity of 1.9–2.0 was treated with samples and centrifuged again at 2500 rpm for five minutes. With a 150 µm mesh, the samples were sieved to remove the plant fragments and coarse debris. The resulting pollen residue was filtered through a 10 µm sieve and kept in distilled water until mounted into glycerol jelly on slides for pollen analysis. The macerated sample was washed and mounted in glycerin jelly. The pollen was classified into different classes based on the pollen size, shape, numbers, exine thickness, pores, and colpi. The pollen taxa were identified by comparing them with the regional palynolora reported elsewhere [25,26]. The Tortonian age of the Murree Formation was assigned to the late Miocene, based on the correlation of plant fossils, fossilized fish remains, and mammalian records and its geological age was confirmed [7]. The main morphological characteristics of the pollen grains to be investigated were as follows: dimension, aperture types, sculpturing, pollen diameter, and shape [1,25]. In the 31 samples analyzed, 15 were barren and 16 contained different types of pollen. With the help of optical and scanning microscopy, we described each sample's morphological features of the pollen grains (Figures 4–8).

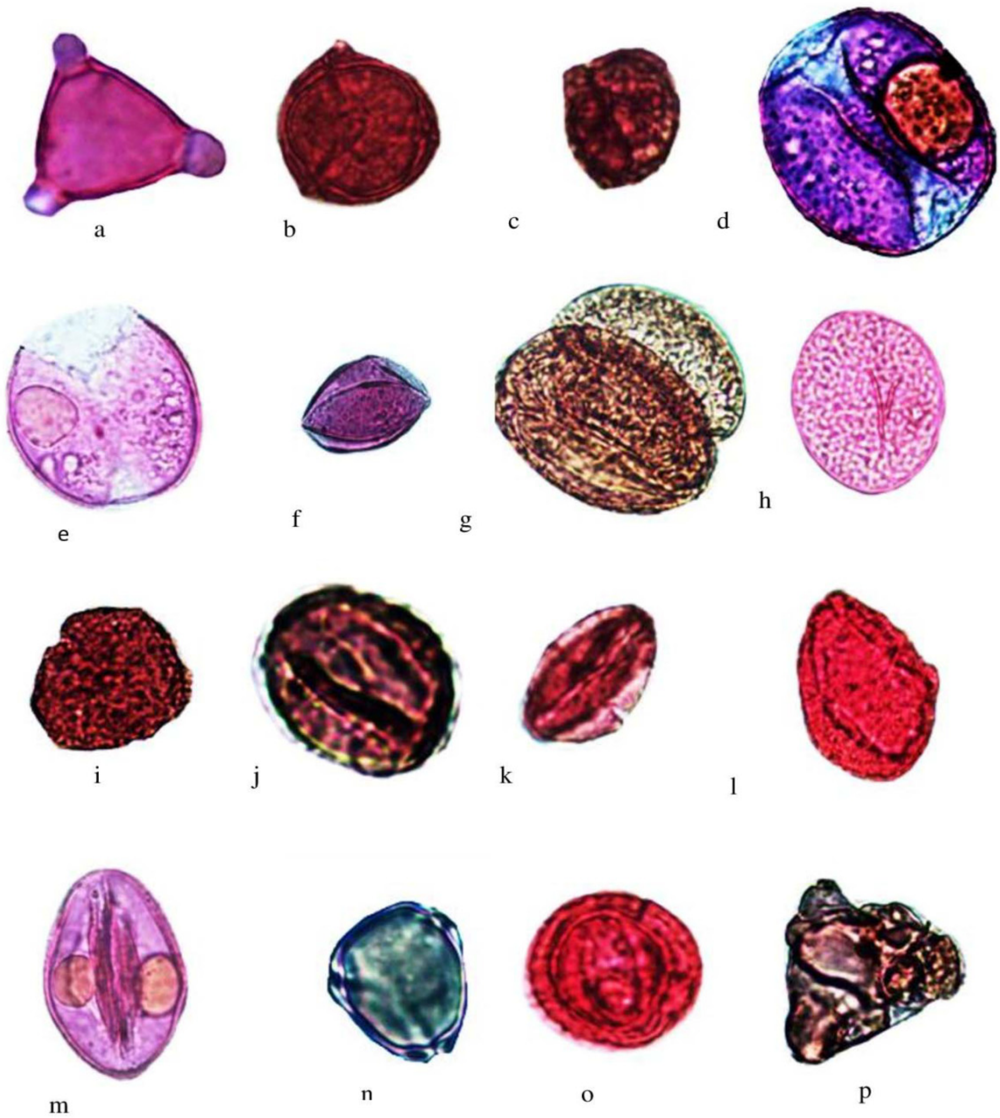

Figure 4. Optical microscopy images of pollen. (**a**) *Gevuina Avellana.* (**b**,**c**) *Sanguisorba minor.* (**d**–**g**) *Pinus* sp. (**h**) *Sparganium* sp. (**i**–**l**) *Quercus* sp. (**m**) *Juniper* sp. (**n**) Myrtaceae. (**o**) *Artemisia* sp. (**p**) *Interporopollenites* sp.

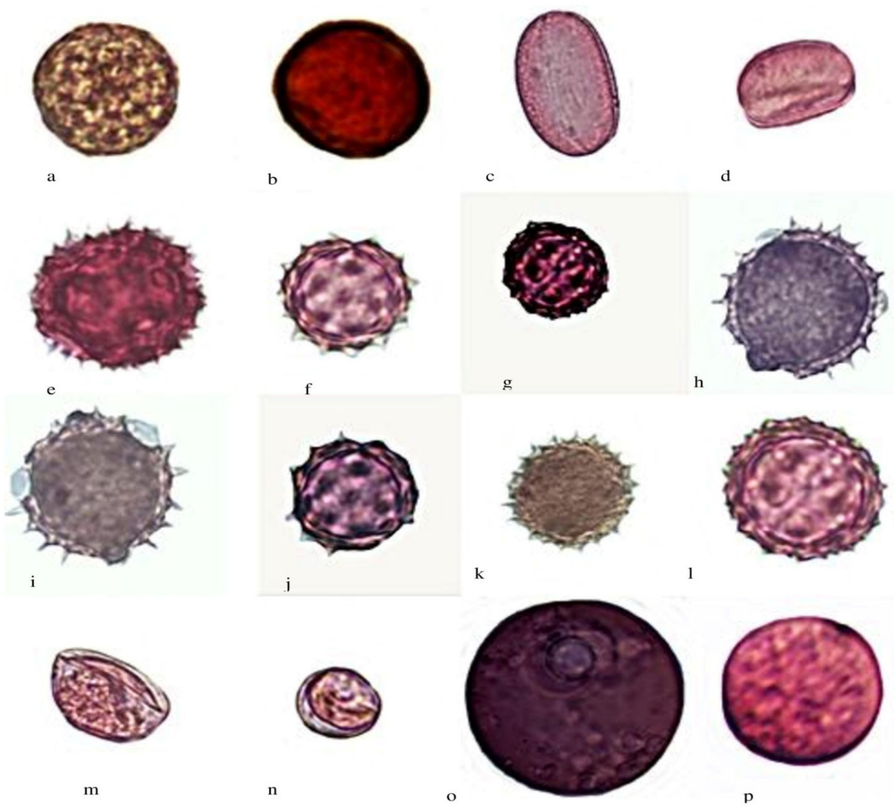

Figure 5. Optical microscopy images of pollen. (**a**) Chenopodiaceae. (**b**) Cupracaceae. (**c**,**d**) Liliaceae. (**e**–**l**) Asteraceae. (**m**–**p**) Poaceae.

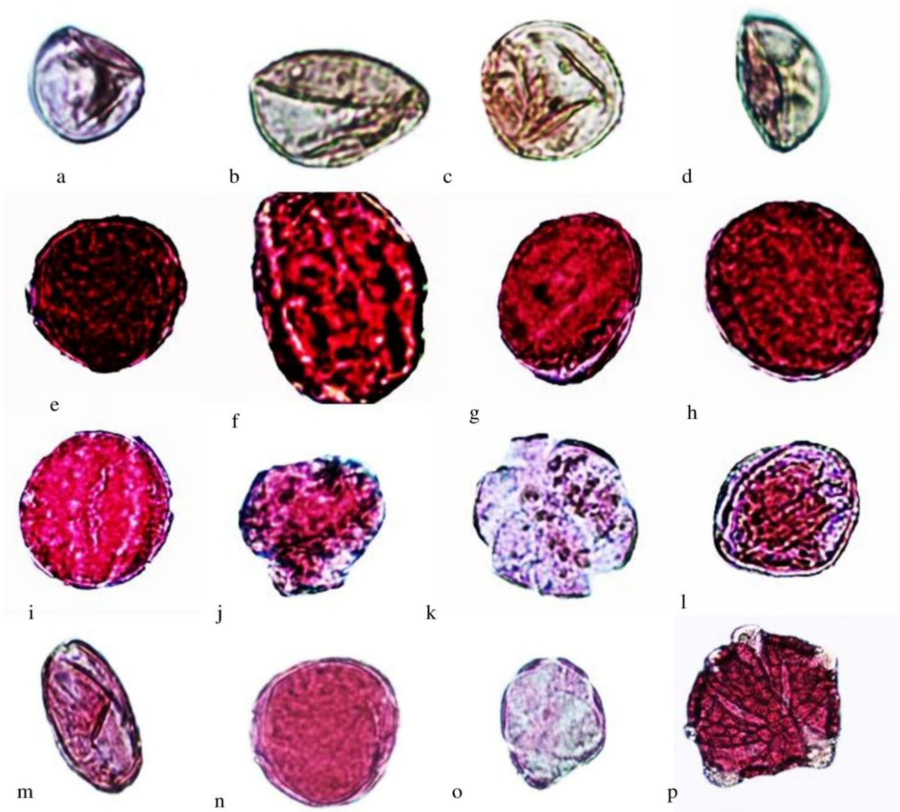

Figure 6. Optical microscopy images of pollen. (**a**–**d**) Poaceae. (**e**–**p**) Lamiaceae.

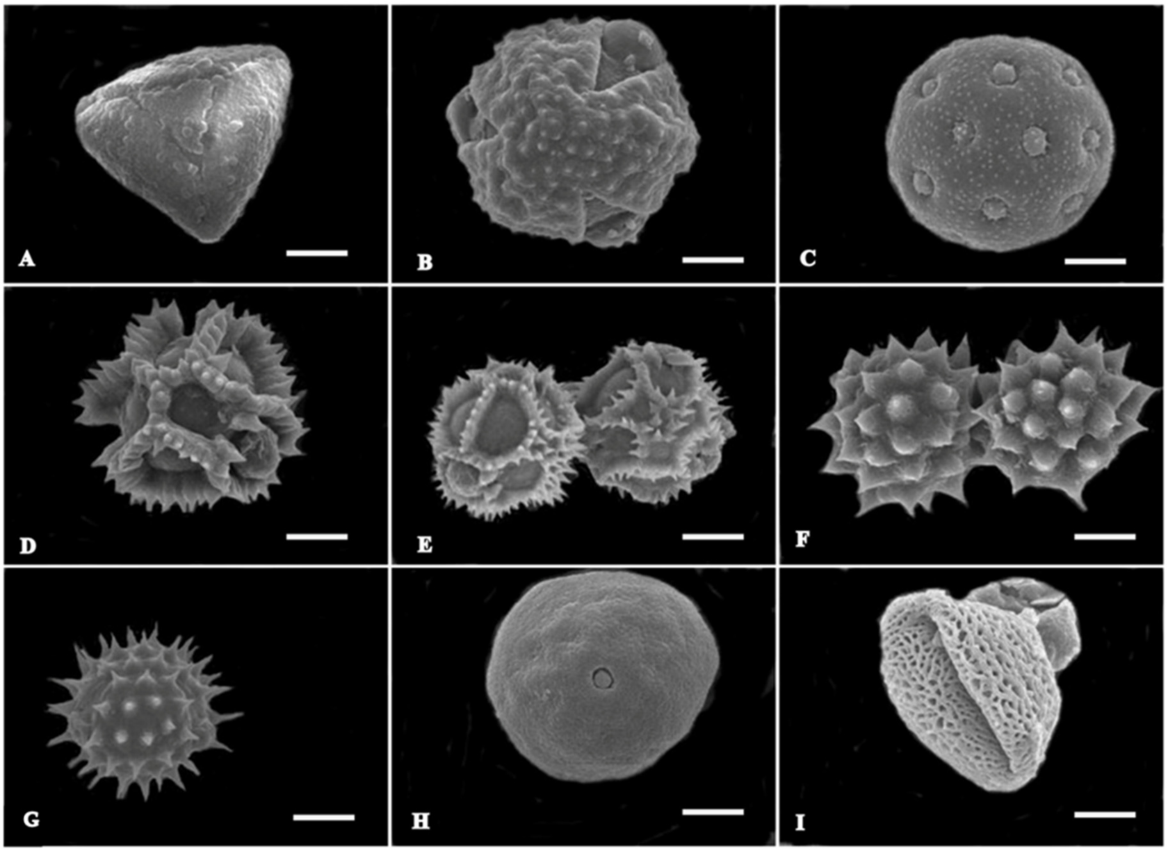

Figure 7. Scanning electron microscopy images of pollen. (**A**) The scale bar represents 5 µm for Myrtaceae, (**B**) 10 µm for *Artemisia* spp. (Asteraceae), (**C**) 5 µm for Chenopodiaceae, (**D**) 5 µm for Cichoriodeae (Asteraceae), (**E**–**G**) 10 µm for other Asteraceae, (**H**) 5 µm for Poaceae, and (**I**) 5 µm for Lamiaceae.

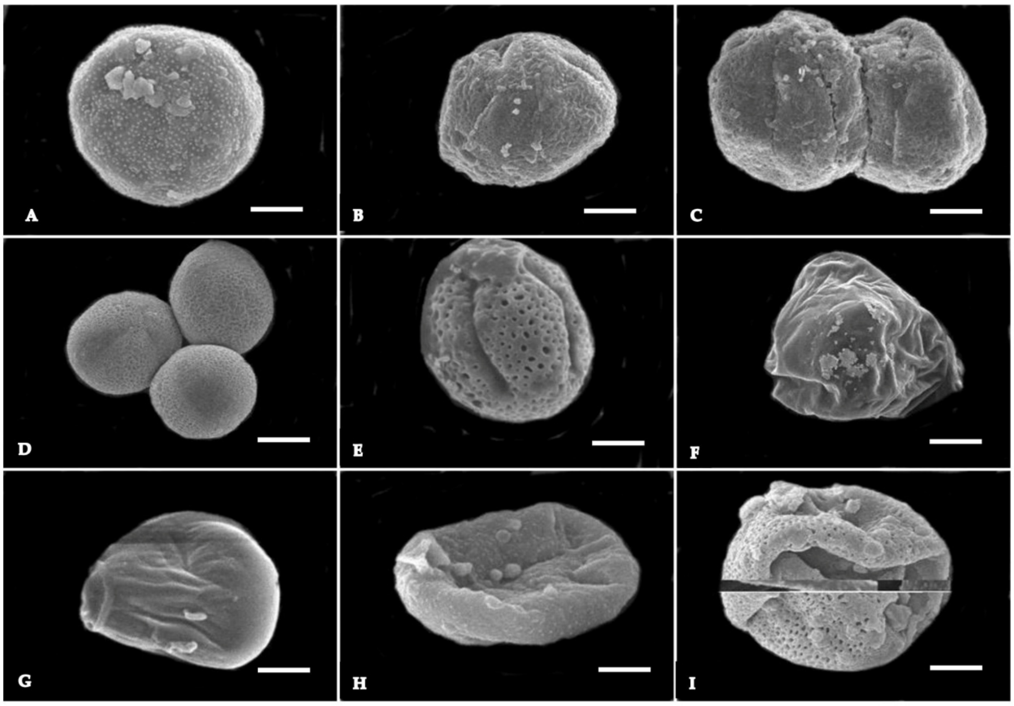

Figure 8. Scanning electron microscopy images of pollen. (**A**–**I**) The scale bar represents 10 µm for Lamiaceae.

2.3. Samples' Identifications

From the late 1970s, the use of standard microscopic techniques has been established as a standard for investigating pollen of fossil plants. Since then, a vast amount of data on the pollen morphology of numerous plant systematic lineages has been gathered. These include books or articles in series that focus on specific genera, families, or species groups. Many smaller publications focused on a single species or multiple species. As a result, prehistoric pollen can be compared using informational descriptions and high-resolution pictures of actual plant material. This makes it possible to identify many fossil pollen grains confidently and gives us the context we need to place the fossil pollen record in a palaeo-botanical and palaeo-biogeographic context. The diagnostic features from the literature under light and scanning electron microscopy were compiled to identify the palynological record of fossil plants. One of the most important criteria is the sculpturing pattern of pollen grains. Different kinds of sculpturing patterns, i.e., psilate, reticulate, and echinate, have been noted in the pollen grains. In addition, the above feature, number of pores, colpi, and spines are the critical features for species identification [19]. The fossil plant source of the pollen grain identification was also made by referencing a collection of modern pollen grains and the published literature. The identification was carried out up to the family, genera, and sometimes to the species level [25].

3. Results

In the present study, 89 pollen types were recorded from 31 samples, of which 48 pollen grains were identified as belonging to 12 different families. Qualitative and quantitative pollen characters are shown in tabular form (Tables 1 and 2) and micrographs are shown in Figures 4–8. The results of the present study showed that the morphological features of pollen were sufficiently variable to be considered helpful for the classification at the species level. The fossil palynomorphs were investigated using optical and scanning electron microscopy.

Table 1. Micromorphological characteristics of pollen—qualitative.

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	*Artemisia* spp.	Spheroidal	-	A	Regulate	A

Table 1. *Cont.*

S. No	Species/Taxon/Family	Pollen Shape	Pollen Type	Colpi/Pore	Ornamentations	Spines
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	Lmiaceae	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

Note: P = Present; A = Absent.

Table 2. Quantitative features of fossil plants pollen from the 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	*Artemisia* 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	-	-

Table 2. *Cont.*

S. No	Species Name	Exine Thickness (µm)	Pollen Diameter(µm)	Colpi Length(µm)	Colpi Width(µm)
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

Approximately 60% of the pollen grains from the study area were identified. An investigation of pollen in thin sections was difficult because the identifications can be made complicated by unfavorable cuts (and damages) through the specimens. The specimens were compared with Pakistan's extinct and modern plants in shape, size, sculpturing pattern, exine thickness, and aperture morphology. The species were identified using microscopic techniques based on morphological features.

3.1. Proteaceae

Gevuina avellana pollen was tricolporate, triangular, isopolar, and radially symmetrical with psilate ornamentations; the exine thickness was 2.25 µm; the pollen diameter was 25.5 µm; the colpi length was 7.00 µm; the colpi width was 6.25 µm.

3.2. Rosaceae

An unidentified genus and species belonging to Rosaceae is listed. The pollen was spheroidal, suboblate, striate micro-perforate, and tricolporate; the exine thickness was 4.00 µm, and the pollen diameter was 38.5 µm.

3.3. Pinaceae

Pinus pollen was circular, subprolate, peroblate, monoporate, angular, dicolporate, psilate, and regulate. The exine thickness was 1.25–3.5 µm; the colpi length was 17.25 µm; the colpi width was 17.25 µm; the pollen diameter was 55.75–69.5 µm.

3.4. Typhaceae

Sparganium pollen was circular, monoporate, and psilate; exine thickness was 2.75 µm and the pollen diameter was 38.00 µm.

3.5. Fagaceae

An unidentified genus and species belonging to Fagaceae is listed. The pollen was inter-angular, prolate-spheroidal, prolate, tricolpate, psilate, and regulate. The exine thickness ranged from 1.25 to 3.25 µm; the pollen diameter was 21.00–32.25 µm; the colpi length was 7.00 µm; the colpi width was 6.25 µm.

3.6. *Cupressaceae*

The *Interporopollenites* pollen is inter-semilobate and psilate; the exine thickness was 1.25 μm and the pollen diameter was 40.25 μm. *Juniperus* pollen was prolate and psilate; the exine thickness was 3.5 μm and the pollen diameter was 56.5 μm. *Cupressaceae* sp. (unidentified species) pollen was spheroidal and psilate; exine thickness was 3.25 μm and pollen diameter was 26.00 μm.

3.7. *Myrtaceae*

Unidentified genus and species belonging to Myrtaceae. Pollen angular, tricolpate, and psilate. The exine thickness was 3.00 μm and the pollen diameter was 23.00 μm.

3.8. *Asteraceae*

Artemisia pollen was spheroidal and regulate; the exine thickness was 1.5 μm and the pollen diameter was 22.5 μm. An unidentified genus and species belonging to the subfamily Cichorioideae were found. The pollen was spheroidal and echinate; the exine thickness was 3.5 μm and the pollen diameter was 22.75 μm. While the rest of the pollen is spheroidal, prolate-spheroidal, circular, and tricolporate; the exine thickness ranged from 3.25 to 4.00 μm and the pollen diameter ranged from 17.25 to 38.5 μm.

3.9. *Chenopodiaceae*

An unidentified genus and species belonging to Chenopodiaceae were found. The pollen was pheroidal, pentaporate, and regulate; the exine thickness was 3.5 μm and the pollen diameter was 25.25 μm.

3.10. *Liliaceae*

An unidentified genus and species belonging to Liliaceae was found. The pollen was prolate, peroblate, and psilate; the exine thickness was 1.00–2.5 μm and the pollen diameter ranged from 32.25 to 42.5 μm.

3.11. *Poaceae*

Unidentified genus and species belonging to Poaceae. The pollen was spheroidal, circular, prolate, subprolate, inter-subangular, monoporate, and psilate; the exine thickness ranged from 1.75 to 2.25 μm. The pollen diameter was 16.50–40.00 μm. The colpi length was 7.00 μm and the colpi width was 6.25 μm.

3.12. *Lamiaceae*

An unidentified genus and species belonging to Lamiaceae was found. The pollen was prolate-spheroidal, semi-angular, angular, circular, and psilate. The exine thickness was 1.25–3.25 μm; the pollen diameter was 30.25–43.00 μm; the colpi length was 7.00 μm; the colpi width was 6.25 μm.

4. Discussion

In the present study, 48 species were reported belonging to 12 different families. In this research, most of the pollen belongs to families of angiosperms: Lamiaceae, Poaceae, Asteraceae, Liliaceae, Cupressaceae, Pinaceae, Fagaceae, Typhaceae, Myrtaceae, Rosaceae, Chenopodiaceae, and Proteaceae (Table 3). Optical and scanning electron microscopy revealed the qualitative and quantitative characteristics of pollen, including the exine thickness, pollen diameter, colpus length, and width. Different pollen shapes were examined, i.e., triangular, circular, spheroidal, prolate, suboblate, subprolate, inter-subangular, and semi-angular. Three types of exine ornamentations were investigated, i.e., psilate, reticulate, and echinate. Similarly, different pollen types were noted, such as tricolporate, monoporate, dicolpate, and pentaporate. In this study, the majority of the species investigated were previously reported in many other regions, i.e., South Asia, Africa, Europe, and America. The contribution of the present work can be considered very important in the understanding of the paleoflora of the study area [28,29]. The existence of pollen in the study area and its identifications is a very reliable tool for the reconstruction of the palaeo-environment and palaeoecology of the area [30]. The floral record of Miocene strata provides a better source as an indicator of the environment.

Table 3. Pollen category, families, taxa, and their 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	*Artemisia* 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

The study of fossil pollen records from the Miocene Epoch sedimentary strata was discussed comprehensively using palynological analysis from micro-fossils [22]. The *Quercus* pollen of Miocene strata was compared with that of the deciduous forest of China based on scanning electron microscopy showing uniformly fine granule, regulate, scabrate-verrucate, and rod-like elements of exine sculpturing [30]. The presence of *Quercus* in the study area revealed a warm temperate climate, and *Pinus* indicates a temperate climate. Cupressaceae, Lamiaceae, Fagaceae, and Rosaceae were investigated in other coeval excavations showing accordance with the present results. In the late Miocene–Pleistocene, mixed forests were described for the Yunnan Plateau of China, particularly coniferous plants belonging to the genus *Abies* and *Picea* pollen [31]. Asteraceae, Poaceae, Fabaceae, Cupressaceae, Liliaceae, Myrtaceae, and Pinaceae were studied in the palynological study of the late Miocene from Siwalik sediments of Uttar Pradesh, which also supports the present findings for evidence in the fossil records of pollen and provides information on the presence of minimal rain with a warm, humid climate in Southeast Asia, such as India and China [32–37]. An early Miocene study on the Dulte Formation was carried out to determine the linkage of palynology and palaeoecology [23,38]. Using combined optical microscopy and SEM, the pollen morphology of Miocene flora for Bignoniaceae, Myrtales, and Lamiales has been reported and showed variations within different palynological features, which are useful for species classification and identification [39]. The formation has marine and tidal influences showing that the vegetation grew near coastal areas, such as salt marshes or mangroves [28]. The indication of the palaeo-environment appears to be in accordance with a previous study [12].

Pollen grains of Asteraceae today were recorded as having tricolporate and were spheroidal, echinated, and zonocolporated [40,41]. In the present study, the Asteraceae pollen was reported to be spheroidal, prolate-spheroidal, and with echinated sculpturing.

A palynological study of the current Poaceae members showed a monoporate-diporate aperture, aerolate and scarbate tectum, while in this study, they were mostly spheroidal, subprolate shapes and inter-subangular and psilate ornamentations [40]. Climatic variations occurred during the Miocene and were responsible for the development and spreading of grasses. Similarly, the past pollen record of Pinaceae in the area was monad, oblate, and nearly circular with narrow attachment alveolate structuring and vesiculate-bisaccate, while in this study, it had a bisaccate, circular-angular shape, and with psilate-regulate exine sculpturing [41]. *Quercus* pollen grains were eutectate, prolate, sculpturing perforate, microverrucate, microregulate (scanning electron microscopy), and scabrate (optical microscopy), while basic sculpturing units were rod-shaped [42]. Several species of *Ocimum* (Lamiaceae) originated from India and then migrated towards the east and west. The palynological record of fossil pollen noted in the literature was mainly hexacolpate, as in the present study with reticulate exine ornamentations. The biogeographical history and phylogeny of other Lamiaceae originating from China and spreading towards the Southwest are closely related to the present results [43,44]. A palaeoecological record of *Sanguisobra minor* (Rosaceae) from the late Eocene-late Miocene was reported from southern central Anatolia [45]. The present study provides data about a larger distribution area with respect to what was previously thought [45]. The Miocene epoch of Pakistan is very important because of the significant environmental changes, but the fossil vegetation records were investigated. The Miocene Murree Formation palynoflora in relation to the palaeoclimate of Pakistan were compared with the modern flora with the fossil record in the depositional environments in China [46,47]. The pollen record for the late Miocene epoch of the Tibetan Plateau was recorded, showing the climatic changes and vegetation distribution briefly as with the present results [48]. *Sanguisorba minor* was dominant in the present investigation showing two different pollen types, while data about the genus *Pinus* showed four distinct pollen grains. Lamiaceae can also be considered dominant, with 12 species attributed on the base of the pollen grains, followed by nine Asteraceae, four Poaceae, and two Liliaceae, while the remaining families contain only one pollen grain. *Ocimum basilicum* had the highest exine thickness (4.25 µm), corresponding to the other literature data. Regarding the pollen diameter, *Pinus* had a maximum value of 69.5 µm, whereas the pollen grains attributable to Asteraceae had a minimum value of 17.25 µm on average. The fossil record of Asteraceae from the Miocene of Patagonia has been reported as being echinated and tricolporated in nature, whereas the current Asteraceae analyzed pollen grains were circular, tricolpate, and echinate exine ornamentations having high thickness [27]. *Gevuina Avellana* (Proteaceae) is considered the oldest species, having psilate exine ornamentations, and radially symmetrical and tricolpated pollen.

This study showed that the paleoflora from Murree formation in the foothills of Margalla provide important information on the paleo-environmental changes in the study area. Fossil-based floral studies were neglected in Pakistan, despite being ecologically and stratigraphically very important, and have never been explored in the proposed study area [29]. Additional research to combine findings from many proxies, including plants and isotope data, is required to comprehend the palaeoclimate history of this region. This type of study will be very important for the paleobotany, paleo-environment, and biogeography of Pakistan, being the first in the investigated area. In addition, such a study helps understand the vegetation origin, the effects of climatic changes with time, and in solving taxonomic problems [49,50].

5. Conclusions

Palynological studies of fossil plants from the Miocene Murree Formation of Pakistan were dominated by angiospermic pollen. This study examined the past vegetation's record, its correlations with the past climate, and its reconstructions. Using optical and scanning electron microscopy, various morphological features were examined, which helped in species identifications. Pollen morphological features, such as pollen size, shape, exine ornamentations, and symmetry, are important for species identifications in plant taxonomy and systematics. The overall results of the Murree Formation are similar to what was previously reported, but some variations in the qualitative and quantitative characteristics of pollen were observed. Further studies to identify the species and genus using advanced microscopic techniques, such as transmission electron microscopy (TEM), are recommended.

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