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**Research Paper**

# Paleopalynological analysis of Primulaceae family evolution during the New Holocene period in Elbasan, Albania

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**ABSTRACT**

This paper presents pioneering paleopalynological data on the Primulaceae family during the New Holocene period in Elbasan, Albania. Fossil pollen was extracted from soil deposits spanning the last 20 centuries, shedding light on the evolutionary trajectory of Primulaceae plants in the region. Soil samples of approximately 0.5 kg, retrieved from depths of 4 m to the surface, were collected at five stations in the Elbasan area using a dry rotary drilling probe between October and November 2023. The Holocene epoch was a critical period of significant shifts in climate and ecological conditions, and understanding plant evolution during this era provides insights into environmental dynamics and human impact on vegetation. A total of 754 Primulaceae palynomorphs were identified across all analysed soil samples, indicating a consistent presence throughout the New Holocene period. The abundance and distribution of Primulaceae pollen suggest enduring resilience and adaptation within the local ecosystem, and the study highlights a correlation between changes in vegetation patterns and human cultivation practices, underscoring anthropogenic influences on regional flora dynamics. Human activities, particularly cultivation, emerge as key drivers of vegetation dynamics in the Elbasan region, highlighting the intertwined relationship between human activities and plant evolution.

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## 1. INTRODUCTION

This study reveals the palynological characteristics of New Holocene deposits in Albania, with a focus on the city of Elbasan. Paleopalynology, a subfield of palynology, is an interdisciplinary discipline with strong ties to geology and the biological sciences, particularly botany; it examines pollen and spores deposited in organic substrates and studies both living and extinct palynomorphs [1], [2]. Because the exine, or outer wall, of pollen grains is highly resistant, pollen can be preserved for long periods in subsurface layers [3], [4]. Research on ancient pollen can contribute significantly to the history of plant use and cultivation, the subsistence of our ancestors, and the development of agriculture [5], and aids in the reconstruction of Holocene stratigraphy, palaeoclimate, and palaeoflora [4], [6], [7], [8], [9]. In this study, we examine the distribution of vegetation during the New Holocene period and the factors that have influenced the transformation of the flora, with a focus on the evolution of the Primulaceae family [1], [2], [6], [7], [8], [9], [10].

## 2. RELATED WORK

To our knowledge, no comparable studies have been conducted by domestic or international researchers on the fossil pollen of Primulaceae in the Holocene deposits of the study area; the microfossils of this family have therefore not previously been studied in Elbasan [6], [7], [8]. The paleopalynological data gathered can contribute to understanding the history of Primulaceae use and cultivation, as well as the origin of the vegetation [6], [10], [11]. The main goal of this study was to establish a relationship between the quantitative fossil pollen data and the changes that the Primulaceae family has undergone over time.

## 3. METHODOLOGY

Examining and analysing fossil pollen makes it possible to assess how the natural environment has changed and how humans have influenced this change [12], [13]. Paleoclimatic and palaeoecological studies of the Quaternary, and consequently the Holocene, rely mostly on paleopalynological analyses, which have a high degree of accuracy [14], [15]. The physico-chemical make-up of pollen allows it to be preserved in, and extracted from, the soil sediments in which it is deposited; almost all techniques are based on the physical and chemical processing of about one gram of soil sediment [1], [4], [6], [7], [8]. The fossil pollen extracted is then subjected to qualitative and quantitative analysis.

In October and November 2023, 16 soil samples were collected in the field at 0.25 m intervals, from depths of 0.25 to 4 m. Every sample contained a sizeable amount of organic matter, indicating that fossil pollen analysis could proceed. Primulaceae palynomorphs were counted by light microscopy at 1000× magnification.

### 3.1 Erdtman Acetolysis Method

Samples were processed using the Erdtman acetolysis method [16], [17]. First, 1 $cm^3$ of soil was combined with 10 mL of 10% KOH. The material was then treated with an acetolysis mixture of acetic anhydride ($(CH_3CO)_2O$) and sulphuric acid ($H_2SO_4$) in a 9:1 ratio. After washing with distilled water, the residue was neutralized and, after acetolysis, centrifuged at 3000 rpm for three minutes. The acetolysis method is widely used in paleopalynology because it gives a clearer view of spores and pollen during microscopic observation than other methods [6], [7], [8], [16], [17].

### 3.2 Mounting of the Preparations

The preparations were mounted in glycerine jelly prepared following the Kisser method [18], using 50 g of gelatine, 175 mL of distilled water, 150 g of glycerine, and 7 g of phenol crystals. After the edges of the cover slip were sealed with paraffin, the slides were ready for use and storage after three days.

## 4. RESULTS AND DISCUSSION

The number of Primulaceae palynomorphs by depth and the total number for the family are given in Table 1.

Table 1. Primulaceae Palynomorphs according to Depth

| Sample | Years AD | Depth (M) | Primulaceae Palynomorphs |
|---|---|---|---|
| 1 | 0 | 4 | 31 |
| 2 | 100 | 3.75 | 34 |
| 3 | 200 | 3.5 | 36 |
| 4 | 300 | 3.25 | 36 |
| 5 | 400 | 3 | 38 |
| 6 | 500 | 2.75 | 40 |
| 7 | 650 | 2.5 | 46 |
| 8 | 800 | 2.25 | 47 |
| 9 | 950 | 2 | 52 |
| 10 | 1100 | 1.75 | 49 |
| 11 | 1250 | 1.5 | 49 |
| 12 | 1400 | 1.25 | 50 |
| 13 | 1550 | 1 | 58 |
| 14 | 1700 | 0.75 | 61 |
| 15 | 1850 | 0.5 | 59 |
| 16 | 2000 | 0.25 | 68 |
| Total | | | 754 |

The maximum number of Primulaceae palynomorphs (68 per sample) was obtained at a depth of 0.25 m and the minimum (31) at 4 m. Except for samples 10 and 15, which show a single slight decrease (at 1.75 and 0.5 m), the number of Primulaceae palynomorphs increases almost continuously from the bottom to the surface, with sharp increases in samples 7, 9, and 13. In total, 754 Primulaceae palynomorphs were counted (Table 1); a representative Primulaceae pollen grain is shown in Figure 1.



Figure 1. Primulaceae Pollen Grain

Climate change can affect how land is used, and human societies can have complex effects on the environment [19]. The data indicate a discernible upward trend in Primulaceae from depth to the surface,

suggesting that the increasing representation of the family should be attributed to human cultivation of its plants. A higher quantity of fossil pollen was also found in the surface samples than in the deep samples, which may partly reflect the better preservation of more recent pollen. Notably, sample 9, at a depth of 2 m, shows a sudden increase in Primulaceae fossil pollen. Because a review of the literature found no significant climatic events in the period around 800–900 AD that might have influenced vegetation change, we relied on the medieval history of Albania [20], which relates this period to devastating wars during the Bulgarian and Serbian occupations, resulting in a great decrease in population and the loss of the city's former economic importance.

The Primulaceae, commonly known as the primrose family, are herbaceous and woody flowering plants found mainly in the mountains of the northern temperate zone, comprising over 1000 species in 22 genera, including many popular garden plants and wildflowers [21], [22]. Despite the paucity of fossil evidence, the age of the crown group has been estimated at 46–61 million years [23], [24]. Many ornamentals of economic significance belong to the family, including Androsace (rock jasmine), Cyclamen, Lysimachia (loosestrife), and Primula (primrose) [25]. Because of their great ornamental value, many species of the family, one of the top three taxa for horticulture worldwide, have been widely grown for hundreds of years [24], and they are also used in cut-flower floriculture [26].

Many Primulaceae genera are widely grown as ornamental plants in public and private spaces, especially in Elbasan, often called the "City of Flowers" of Albania. The presence of Primulaceae palynomorphs in every sample and their increasing trend from deep to surface samples suggest that humans have had a significant influence on these plants, primarily through the family's long history of cultivation in Elbasan, supported by the consistent presence of these plants over time. Elbasan is now home to a variety of plants, mainly olive plantations and commonly farmed fruit trees, and to a very rich flora and vegetation, and ecological and human factors have strongly influenced pollen dispersal. The quantitative fossil pollen data for Primulaceae thus indicate the direction in which the family's plants have evolved over time.

## 5. CONCLUSION

- Primulaceae palynomorphs become more prevalent from depth to the surface.
- Members of the Primulaceae family are present in all the examined samples.
- Since Elbasan has not experienced significant climatic changes that could have influenced the change in flora over time, human influence is considered responsible for it.

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### Authors Contributions Statement

| Name of Author | C | M | So | Va | Fo | I | R | D | O | E | Vi | Su | P | Fu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

### Conflict of Interest Statement

No conflict of interest.

### Informed Consent

Not Applicable.

### Ethical Approval

Not Applicable.

### Data Availability

This study draws on publicly available data. No new datasets were created.

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