Comparative Palynological Study of Some Species of Asteraceae (Compositae) and Brassicaceae Family in Albania

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Abstract: - This study aimed to compare the palynological features of some species: *Iberis sempervirens* L., *Bunias erucago* L. (Brassicaceae family), and *Aster tripolioum* L., subspecies *pannonicus* (Jack.) Boo. (Asteraceae family). The botanist Ermelinda Gjeta determined these species. I have prepared five slides for each species using acetolysis and the basic fuchsine method. The study of these species' palynological features was done for the first time in Albania. We have compared the features with those of *Bunias orientalis* L. and *Aster albanicus* Degen, taken from local and foreign literature. According to the results of this study, the pollen grains of *I. sempervirens* and *B. erucago* were tricolpate inaperturate; in the equatorial view, the shape of pollen grains was elliptic to circular, while in the polar view, the shape was circular. The pollen grains of *A. tripolioum* L., subspecies *pannonicus* (Jack.) Boo. were tricolporate; in equatorial view, the shape was spheroidal. The exine has an echinate sculpture and was bilayered in *A. tripolioum* L., subspecies *pannonicus* (Jack.) Boo., while in *I. sempervirens* and *B. erucago*, the exine was reticulate.

Key-Words: - pollen, *Asteraceae*, *Brassicaceae*, echinate, reticulate, colporate, colpate.

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1 Introduction

According to Albanian Flora, the Brassicaceae family includes about 59 genera and 191 species and subspecies [1], while the Asteraceae family, according to recent studies, contains about 388 species [2]. These two families include annual, perennial, or bush plants**.** The plants of these two families can be honey and medicinal, but they can also cause allergies in some people who are sensitive to the allergic factor.

With botanical and palynological importance, these two families include the genera *Iberis* and *Bunias*, respectively, with the species *Iberis sempervirens* L., *I. pruitii* Tineo, *I. umbellata* L., and *Bunias erucago* L., and the *Aster* with eight species [*Aster squamatus* (Spreng.) Hieron, *A. amellus* L., *A. alpines* L., *A. bellidiastrum* L. (Scop.), *A. tripolioum* L., subsp. *pannonicus* (Jack.) Boo., *A. sedifolius* L., *A. albanicus* Degen, *A. linosyris* (L.) Bernh.] as well as many other subspecies.

Many scientific works in palynology, both foreign and local literature, are related to the representatives of the genus *Iberis*, *Bunias*, and *Aster*. From them, it follows that, in general, the representatives of the genus *Iberis* and those of the genus *Bunias* have tricolpate pollen grains, and the exine layer appears reticulate, while representatives of the genus *Aster* have tricolporate pollen grains and echinate exine, [3], [4], [5], [6], [7], [8], [9], [10], [11], [12], [13], [14], [15].

The object of this study was the examination of the palynological characteristics of the pollen grains of the species *I. sempervirens*, *B. erucago,* and *A.*

tripolioum L., subspecies *pannonicus* (Jack.) Boo., taken in different habitats of our country.

The purpose of this study was:

- To study for the first time the morphological characteristics of the pollen of Albanian plants belonging to the Asteraceae and Brassicaceae families;
- We refer to Albanian palynological literature sources [11] and [14] to determine the similarities and differences of their palynological features compared to other species.

2 Material and Methods

To provide the study material, we have conducted several expeditions in groups in different areas of Albania, such as the Shebenik National Park, the Librazhdi area, the Krasta hills around the city of Elbasan, and the Butrint area in the Saranda region.

Albania has a subtropical Mediterranean climate, which, together with its geographical position, allows for a high biodiversity of flora and fauna, [16].

Shebenik National Park is located 300-2200 m above sea level and is of great scientific, economic, and cultural importance. On 20.05.2023, we found *Iberis sempervirens* on the roadside near a rocky massif in this park.

Elbasan, located in the center of Albania, is surrounded by hills, particularly the Hills of Krasta. These hills leave rich, herbaceous, shrubby, and woody vegetation. On 06.05.2023, on the side of the road to the top of Krasta, we found *Bunias erucago*.

A. tripolioum L., subspecies *pannonicus* (Jack.) Boo. is found on the edge of the Vivari Channel, located in Butrint, near Ali Pashë Tepelena castle, in Saranda region, Albania, on 10.09.2023**.**

This channel is 18 km away from the city of Saranda. The Vivar Channel is an essential part of the hydrological network of the Butrint National Park territory as a natural channel through which Butrint Lake communicates with the Ionian Sea. It has a length of about 2.5 km and a depth of around 6 m, [17].

The channel is the migratory fish's main circulation route and constitutes a unique ecosystem. Since 2003, the Vivar Channel, like the rest of the Butrint Lagoon, has been declared a "Ramsar Site" for its distinctive values and biodiversity.

Butrinti Lagoon is one of the most fascinating valleys in Albania, and Butrinti Lake has large fish stocks, [18].

Knowledge of the palynomorphological features of pollen grains has practical applications in various scientific fields, such as:

- Melissopalynology;
- Allergopalynology;
- Paleopalynology, also,
- Forensic palynology.

As high as possible, this knowledge allows us to determine the floral origin of honey (monofloral or polyfloral), identify allergenic pollen grains, contribute to criminal investigations, track plant evolution over time, and demonstrate the tangible impact of our research, [19], [20], [21].

During observation under a microscope, we can count the pollen grains belonging to a particular species that lived in a specific area, thus forming a pollen diagram. This diagram allows us to discover the environmental situation before and the human impact on that area, e.g., when deforestation occurred, what the land use was, what the crops were used for, or what the possible climate changes in the region were, [22].

The literature shows that the image of pollen grains also helps in many aspects related to forensic palynology. The structure of pollen and spores is highly resistant to any possible external environment, including heat and cold, washing, staining, and degradation. Pollen grains can remain preserved for many years and can even serve to solve crime scenes after a very long period, [23].

With the pollen of these plants, five slides were prepared for each species using two study methods: acetolysis [24] and the basic fuchsine method [25]. We used the Kisser method to fix pollen grain slides [26]. For each species, about 31 measurements we made to determine their dimensions: the length of the equatorial axis, the length of the polar axis, the length of mesocolpi, the width of the colpi, the diameter of porus, the thickness of the exine, the length and width of the end of the spines, etc. The sculpture of the exine layer has also been studied. The terminology used is firmly based on that recommended by established sources such as [27] and [28].

The study of pollen grains' morphological characteristics uses a powerful tool: the biological microscope (Motic BA310 Series LED-Digital). With magnifications of x100, x400, and x1000, this instrument allows us to delve into the intricate details of pollen grains. The study is accompanied by photos of the species taken by Nikoleta Kallajxhiu in the place where they are found, as well as microphotographs of the pollen grains in different views, equatorial and polar views. This study brings

for the first time in the Albanian literature the palynomorphological description of the pollen grains for the species *I. sempervirens* L., *B. erucago* L. (Brassicaceae), and *A. tripolium* L., subspecies *pannonicus* (Jack.) Boo. (Asteraceae).

3 Results and Discussion

Family: **Brassicaceae**
Genus: ***Iberis* L.**
***Iberis sempervirens* L.** - Perennial Candytuft
It is a procumbent woody shrub with white petals that are rarely flushed. This unique plant, *I. sempervirens* L., is widespread in subalpine rocks and stony places and is easily found in Shebenik National Park. Its flowering period is around June-August.

The pollen grains of *I. sempervirens* were monads, with radial and isopolar symmetry. They were tricolpate pollen grains. It looks obvious in the photos taken (Figure 1). The shape of the contour was spheroidal (P/E = 1.02). The diameter of pollen grains varies from 20-22.5 (21.87) µm ± 0.42. The web cellules reach up to 1.25 µm. The distance between two colpi varies from 14.75-15.5 (15.42) µm ± 0.26. The length of colpus varies from 10.25-11.5 (10.95) µm ± 0.16, and the width was, on average, 4.5µm. The exine of the pollen grain was bilayered. Its sculpture was reticulate.

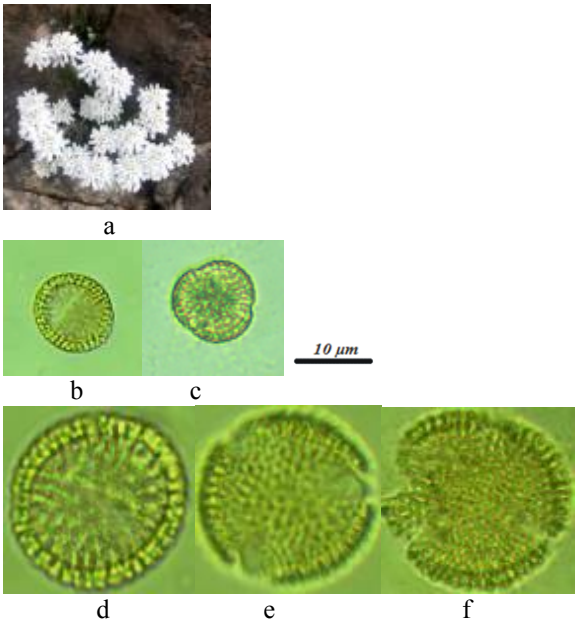

Fig. 1: Photo of *I. sempervirens* (a - photo of species in its habitat; b – microscopic images of pollen grains in equatorial view x400, acetolysis method; c - polar view x400; d - equatorial view x1000 and e, f - polar view, x1000); photo: Kallajxhiu, N

Genus: ***Bunias* L.**
Bunias L. is a genus of the Brassicaceae family, like the *Iberis* genus mentioned above. According to Albanian flora, it includes only one species, *Bunias erucago* L., [29].

***Bunias erucago* L.** - Common Rocket
B. erucago, an annual or biennial herb, is a familiar sight, standing at 20-60 cm. This hermaphrodite species, pollinated by bees, is evidence of nature's self-fertility. Its yellow corolla adds a splash of colour to the fields, meadows, and wastelands it populated, a common sight in low areas, and the Hills of Krasta, a region known for its diverse flora and unique geological features. Its widespread flowering period, from April to July, ensures that it is a plant that many can connect with [29].

Figure 2 shows that pollen grains were tricolpate monads with radial symmetry, and their polarity was isopolar. The shape of the pollen grains, according to the contour, was spheroidal (P/E = 1.05).

The pollen grains were oval in the equatorial view, while the outline in the polar view was circular. The length of the equatorial axis of the pollen grain varies from 21-29 (24) µm ± 0.25, while the length of the polar axis varies from 21-29 (22.83) µm ± 0.26. Based on the data on the size of pollen grains, it turned out that they were small pollen grains [22]. The colpi were long and reached the pole of the pollen grains. Their width reaches up to 4.1 µm. Mesocolpium (the distance between two colpi) varies from (10-16) 13.5 µm ± 0.20. The exine was bilayered, whereas the ectexine was rather thicker than the endexine. It was equipped with a reticulate surface, and its thickness reached up to 3.5 µm. The exine in the colpi was granulated.

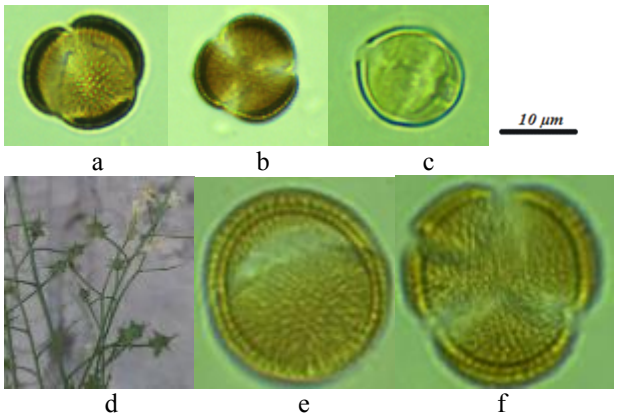

Fig. 2: Images of pollen grains (a, b - microscopic images of pollen grains in polar view x400, acetolysis method; c - pollen grain in equatorial view x400, acetolysis method; d - photo of *B. erucago,* done by Gjeta, E.; e - pollen grains in

equatorial view x1000, acetolysis method and f - pollen grains in polar view x1000, acetolysis method); photo: Kallajxhiu, N

The scientific community widely acknowledges that the global temperature is on a steady rise, accompanied by an increase in rainfall and a shift in overall climatic conditions, [30], [31]. Equally significant, numerous authors have demonstrated the profound and crucial influence of ecological factors and pollen grain processing methods on their size, [32]. This underscores the importance of our research in understanding climate change.

This influence of clima led us to compare the sizes of the pollen grains of *B. orientalis* with those from the literature [14]. The resulting data is presented in Table 1, underscoring the importance of our research findings.

Table 1. Compared values of pollen grains of *B. erucago* with those of *B. orientalis*

Features of pollen grains in µm ± SD	*Bunias erucago*	*Bunias orientalis* [14]
Length of polar axis (P)	(21-29) 22.83 ± 0.26	26-30
Length of equatorial axis (E)	(21-29) 24 ± 0.25	26-30

The data in the Table 1 clearly show that the pollen grains of *B. erucago* were smaller than those of *B. orientalis* in the minimum and maximum values. Based on the literature regarding these pollen grain sizes, we conclude that these pollens were included in the average size group (25-50 µm), [4].

Family: **Asteraceae**
Genus: ***Aster* L.**
Aster tripolioum* L.**, subspecies ***pannonicus (Jack.) Boo. - Sea Aster, Sea-Starwort

This species is an annual or perennial herb and a therophyte/hemicryptophyte plant. The corolla has 10-30 petals, blue or purple, always present. It grows in sandy places with more humidity, mainly by the sea. Flowering: August to September, [29].

The pollen grains of *A. tripolioum* L., subspecies *pannonicus* (Jack.) Boo. were tricolporate, as depicted in Figure 3. The length of the polar axis varies from 24.75-30 (27.75) µm ±1.28 while the length of the equatorial axis varies from 25.25-30 (28.43) µm ±1.4. The shape of the pollen grain was almost spheroidal triangular (P/E = 1.02).

The diameter of the pores varies from 5-7.5 (6.2) µm ± 0.37. The average distance between two colpi (mesocolpium) varies from 18.75-20.25 (19.7) µm. ± 0.20. The exine layer was echinate, and its thickness was almost 2.5 µm. The base of its spines was about 4.75 µm in diameter while they were 2.75 µm long, so to conclude, their base width was more significant than their height.

The results obtained in this study show that the pollen grains of *A. tripolium* subspecies *pannonicus* are included in the medium-sized pollen grains group, [4].

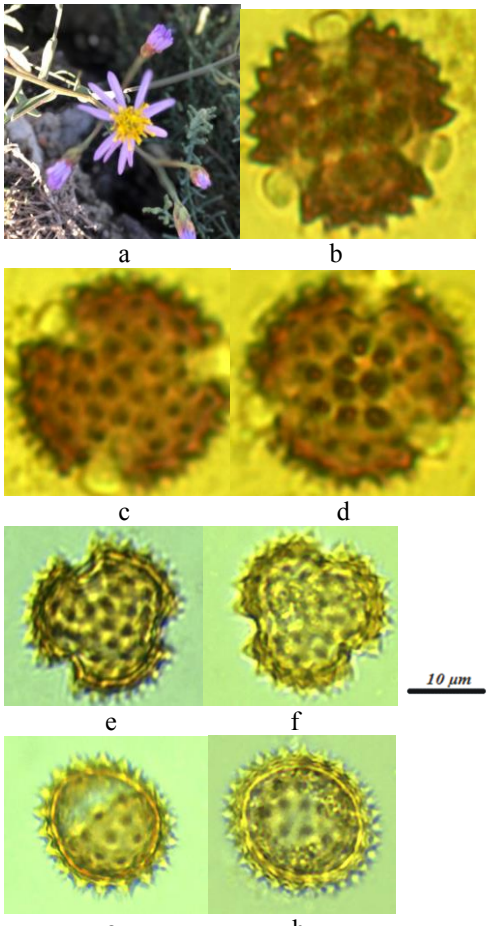

Fig. 3: Images of *A. tripolioum* L. subspecies *pannonicus* (Jacq.) (a - photo of species done in its habitat; b, c, d - microscopic photos in polar view x1000, basic fuchsine method; e, f - polar view x400, acetolysis method; g, h -equatorial view x400, acetolysis method); Kallajxhiu, N

To determine differences and similarities in the palynological features, we have compared the pollen grains of *A. tripolioum* L. subspecies *pannonicus* (Jack.) Boo. to those of *A. albanicus* Degen, found

in the coastal area of Divjaka, Albania, referred to the local literature, [11].

Table 2 presents the minimum, maximum, and average values of the palynological characteristics of *A. tripolioum* subspecies *pannonicus* compared to those of *A. albanicus*.

Table 2. The palynological data of *A. tripolioum* subspecies *pannonicus* compared to those of *A. albanicus*

Features of pollen grains in µm ± SD	*A. tripolioum* L., subspecies *pannonicus* (Jack.) Boo.	*A. albanicus* Degen [11]
Length of polar axis (P)	24.75-30 (27.75) ±1.28	31.2-36.4 (33.95)
Length of equatorial axis (E)	25.25-30 (28,43) ±1.4	31.2-36.4 (34.66)
Mesocolpium	18.75-20.25 (19.7) ± 0.20	19.01
Diameter of pores	5-7.5 (6.2) ± 0.37	4-5 (4.9)
Thickness of exine without spines	2.5	2
Thickness of exine with spines	5.0	5.5
Length of spines	2.5	3.5
Width of spines	4.75	4.07

The data in Table 2 are not only objective but also indisputable. They show that the polar axis (P) of the pollen grain of the *A. tripolioum* subspecies *pannonicus* is consistently more minor than that of *A. albanicus* (precisely the average value 27.75 µm), respectively as an average value of 33.95 µm, as indicated by the length of the polar axis in two other values, (minimum 24.75 µm versus 31.2 µm, maximum 30 µm versus 36.4 µm).

The same results are for the length of the equatorial axis (E), which is consistently smaller (precisely the average value 28.43 µm) than that of *A. albanicus* (respectively an average value of 34.66 µm), as indicated by the length of the equatorial axis in two other values (minimum 25.25 µm versus 31.2 µm, maximum 30 µm versus 36.4 µm).

Figure 4 clearly shows that the pollen grains of the A. *tripolium* subspecies *pannonicus* found in the Butrint area had pores with larger diameters in the minimum and maximum values, as well as in the average values, than the species *A. albanicus* taken in the Divjaka area, which we compared.

Thus, specifically, the diameter of the pollen grains of the *A. tripolium* subspecies *pannonicus* is more significant in all three values than that of *A. albanicus*. Thus, the minimum value is 5 µm versus 4 µm in *A. albanicus*; the average value is 6.2 µm versus 4.9 µm in *A. albanicus*, and the maximum value is 7.5 µm versus 5 µm in the other species.

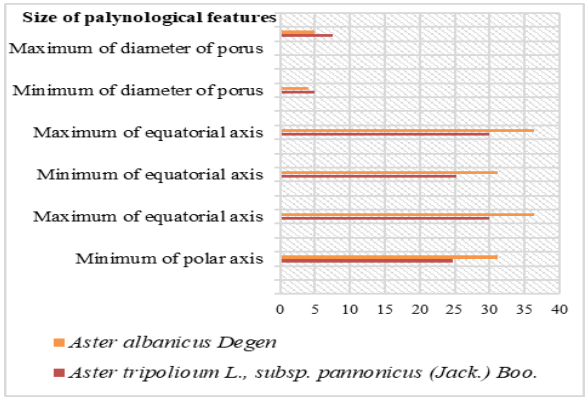

Fig. 4: The graph of minimum and maximum values of pollen grains of *A. tripolium* L. subspecies *pannonicus* (Jack.) Boo., compared those to *A. albanicus* Degen

Another difference in size among the pollen grains of the two species we mentioned is the distance between the two colpi (mesocolpium). This feature is particularly prominent in the *A. tripolium* subspecies *pannonicus*, serving as a key distinguishing feature. As for the exine layer, with or without spines, it had a thickness almost equal to that of *A. albanicus* taken in the Divjaka area.

Spines accompanied the exine in the pollen grains of both species, but those of *A. tripolium* subspecies *pannonicus* were shorter but wider at the base than those of *A. albanicus*.

4 Conclusion

Our comparative palynomorphological study of *Iberis sempervirens*, *Bunias erucago,* and *Aster tripolium* subspecies *pannonicus* is a help in palynology. The data about the palynological features of these species have been described for the first time in the Albanian literature, thus contributing to the identification of the palynological features of many species located in Albania.

This investigation resulted in:

- The pollen grains of *I. sempervirens* and *B. erucago* were tricolpate, while those of *A. tripolium* subspecies *pannonicus* were tricolporate
- Smaller sizes of pollen grains are identified in *B. erucago* compared to *B. orientalis,* and in the pollen grains of *A. tripolium* subspecies *pannonicus* compared to *A. albanicus*
- The exine layer is reticulated in *I. sempervirens* and *B. erucago,* and the colpi were granulated. In the pollen grains of *A. tripolium* subspecies *pannonicus*, an echinate exine accompanied by

spines is identified, but those of *A. tripolium* subspecies *pannonicus* are shorter but wider at the base than those of *A. albanicus*

- The diameter of the pores in *A. tripolium* subspecies *pannonicus* was more significant than that of *A. albanicus*
- As a working group, we recommend that in the future, to assess the environmental impact, we determine the size of the pollen grains of the same plants in other habitats in Albania.
- We will build a database with comparative data on the sizes and palynological features of these plants, based on relevant studies by different authors.
- We are working on creating a pollen atlas with pollens of allergenic plants in Albania and beyond.

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It is an honor to be part of this journey exploring the wonders of botanical science, especially in adding Albania to the map of essential studies done in this field. I want to thank my colleagues who helped me with this research; I could not have done it without them.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

- Nikoleta Kallajxhiu, an expert in Palynology and Allergo-palynology, developed this study to provide essential data regarding pollen grains in Albania. For the first time, an Albanian author is studying these pollen grains. The expedition, led by N. Kallajxhiu, gathered various plant samples from different regions of Albania as the first part of this study. N. Kallajxhiu then prepared the slides using specific methods and examined them under a microscope.
- Ermelinda Gjeta was part of the expedition team and was responsible for identifying the plants as a botanical expert.
- Silvana Turku, also a member of the expedition team, has a keen interest in Genetics in Botany. She plans to develop a genetic study of the plants mentioned in this research.
- Dhurata Valera was responsible for the descriptive statistics.

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Conflict of Interest

The authors have no conflicts of interest to declare.