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# Study of Palynological Polymorphism in Spartium junceum L. Growing in the Tébessa Region

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**...THANK YOU...**



# *Dédicaction*

بعد سنين من التعب، السهر، والدموع...

ها أنا اليوم نوقف على عتبة حلمي اللي تحقق بعد سنوات من الجهد والمثابرة، تحقق الحلم الذي طال انتظاره.

أتوجه بجزيل الشكر والامتنان لعائلتي،

خاصة والدي **حسين غرايبية** ووالدتي **منية شابي**، على دعمهما المتواصل.

كما أشكر إخوتي **نور الهدى، روميساء، علاء، وعثمان** على حبهم وتشجيعهم.

ولا يفوتني أن أعبّر عن امتناني لأصدقائي **أسامة، عبد القادر، نضال، محمد، عبد الرزاق، وسيف** على وقوفهم إلى جانبي.

ولزميلي في التخرج **إسلام حراث** على المشاركة الصادقة في هذا المشوار...

**تقي الدين**



# *Dédicaction*

*I would like to thank my family and friends for every moment I have lived during my university career.*
*Secondly, special thanks to my friends and family, present and absent, HA FW FA CH H LZ , without counting them.*

# Résumé

Ce travail porte sur l'évaluation de la diversité morphologique des grains de pollen chez le Genêt d'Espagne (*Spartieum junceum L*), dans la région nord de Tébessa.

L'étude repose sur la méthode d'Ertman, qui consiste à analyser le rapport entre l'axe polaire (P) et le diamètre équatorial (E), en se basant sur des critères morphologiques précis pour déterminer la forme des grains de pollen.

Les résultats révèlent une diversité de formes palynologiques, avec une prédominance des types sphéroïdal prolate, suboblate et prolate.

D'autres formes, en revanche, ont été observées rarement ou pas du tout au cours de l'étude.

**Mots clés** *: Spartieum junceum L*, pollen, diversité morphologique, Tébessa.

# Abstract

This study focuses on assessing the morphological diversity of pollen grains in Spanish Broom (*Spartieum junceum L*), found in the northern region of Tébessa.

The research is based on Erdtman's method, which involves analyzing the ratio between the polar axis (P) and the equatorial diameter (E), using precise morphological criteria to determine pollen grain shape.

The results reveal a variety of palynological forms, with a predominance of spheroidal prolate, suboblate, and prolate types.

Other forms, however, were observed only rarely or not at all during the study.



**Keywords:** *Spartieum junceum L*, pollen, morphological diversity, Tébessa.

# ملخص

تناول هذا العمل تقييم التنوع المورفولوجي لحبوب اللقاح في نبات (*Spartieum junceum L*)في المنطقة الشمالية من تبسة.

تعتمد الدراسة على قانون (Erdman)، التي تتضمن تحليل النسبة بين المحور القطبي (P) والقطر الاستوائي(E) ، بالاستناد إلى معايير مورفولوجية دقيقة لتحديد شكل حبوب اللقاح.

تكشف النتائج عن تنوع في الأشكال الباينولوجية، مع هيمنة الأنواع الكروية المستطيلة(sphéroïdal prolate) ، وشبه المنبطحة(suboblate) ، والمستطيلة.(prolate)

**الكلمات مفتاحية** : *Spartieum junceum L* :، حبوب اللقاح ، التنوع المورفولوجي، تبسة.

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# Introduction

## *Introduction*

The term biodiversity, a neologism introduced during the 1992 Earth Summit in Rio, has since become widely used, largely due to the ongoing biodiversity crisis and its extensive media coverage **(Prieur, 2015; Wilson, 1992).** Biological diversity encompasses multiple levels and manifests in various forms. It can be considered at the genetic scale, the species level, or within ecosystems **(Heywood, 1995),** and numerous processes contribute to generating this diversity among living organisms **(Purvis & Hector, 2000).**

Natural selection operates across all levels of biological organization, with the individual serving as a crucial unit of selection **(Endler, 1986).** Observable traits such as shape, color, and other morphological features are subject to both natural and sexual selection **(Andersson, 1994).** Consequently, selective processes play a key role in the evolution of morphology, alongside developmental factors that can either constrain or facilitate the emergence of specific forms **(West-Eberhard, 2003).**

This study focuses on analyzing pollen grain diversity in *Spartieum junceum L* a plant growing in the north of Tebessa region.

For the first time, the pollen of these two species has been analyzed using Erdtman's method **(Erdtman, 1953),** which involves measuring the ratio of the polar axis (P) to the equatorial diameter (E), following morphological identification keys to accurately determine pollen shape **(Erdtman, 1969).**

The results reveal a wide range of pollen morphologies characterizing the species under study, including spheroidal prolate, suboblate and prolate forms. However, some forms were very rare such as peroblate, oblate and perprolate.

These findings are detailed in Chapter 3, while the literature review and the materials and methods employed are presented in Chapters 1 and 2, respectively.

Andersson, M. (1994). Sexual selection. Princeton University Press.

# CHAPTER I :
# Literature Review

# I. Pollen

## 1. Definition

Pollen is the male reproductive element of seed plants. It consists of a multitude of microscopic particles contained within the pollen sacs of the anthers, which produce the male fertilizing elements of flowers **(Charpin, 2004).** When mature, the anthers release the pollen.

Each pollen grain is a tiny structure, ranging in size from 5 to over 300 µm. It can be spherical or rod-shaped and has a variable lifespan, lasting from a few minutes to several days. The pollen grain produces male gametes, making it the male gametophyte of seed plants, rather than the male gamete itself **(Marouf, 2007) (Figure 1).**

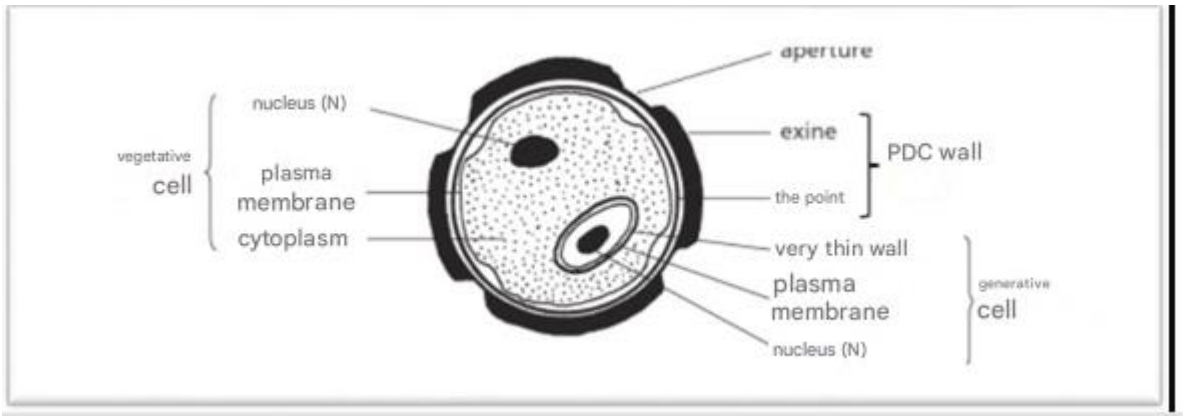



**Figure 1:** The Structure of the Pollen **(tPeycru et *al.*,2010).**

## 2. Microscopic characteristics

- **Shape**

Pollen grains exhibit a wide variety of shapes, including spherical, oval, elongated, triangular, semi-circular, cubic, hexahedral, or pentagonal forms **(Erdtman, 1943) (Figure 2).**

The shape of a pollen grain is determined by the ratio between the dimensions of the polar axis (P) and the equatorial axis (E) **(Pons, 1958).**

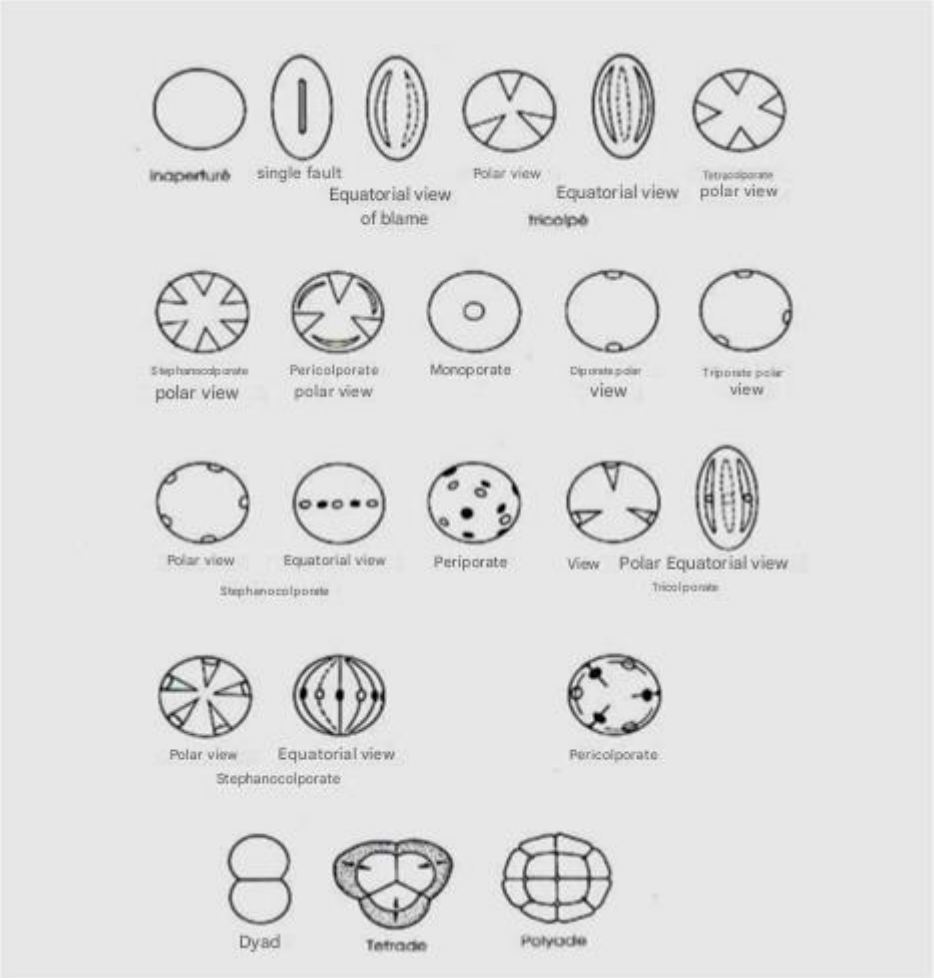



**Figure 2:** Different Pollen Types in Angiosperms Based on the Number and Arrangement of Apertures **(Tschudy & Scott, 1969, in Chateauneuf & Reyre, 1974; Renault-Miskovsky & Petzold, 1989; and Guerin et *al.*, 1993).**

- **Size :**

The size of pollen grains ranges from 5 µm in *Myosotis* to 250 µm in certain gymnosperms, such as fir and spruce **(Laaidi et *al.*, 1997).** Many anemophilous pollen grains measure between 20 and 60 µm **(Charpin, 1986).** Although pollen size can vary depending on the plant's age and maturation conditions, it remains generally consistent within the same species.

The size of pollen is correlated with the structure of the stigma papillae. The equatorial diameter of the pollen corresponds to the spacing of the papillae on the receptive surface, while pollen volume is linked to style length. **(Roulston et *al.*, 2000).**

- **Structure :**

A mature pollen grain typically contains two haploid nuclei: a larger vegetative nucleus and a smaller generative or reproductive nucleus. A double outer layer, known as the sporoderm,

which consists of two distinct walls, encloses it. The outermost layer, the exine**,** is highly resistant, while the inner layer, the intine, forms the endospore.

The size of these structures varies depending on the genus and species, ranging from 15 to 45 microns (thousandths of a millimeter) **(Philippe, 1991) .**

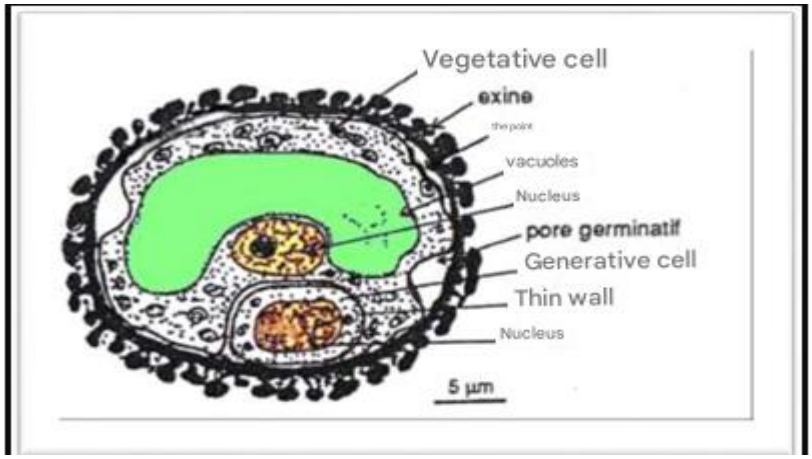



**Figure 3:** The Structure of a Pollen Grain **(Caulien, 2009).**

- **The Exine:** A Protective Outer Shield

The exine is the tough outer layer of a pollen grain, designed to withstand the challenges of dispersal through the air without damage. Its rigidity acts as a protective shield, preventing the grain from being crushed or worn down.

Interestingly, the exine's texture and appearance vary between species, making it an essential tool for identifying different types of pollen. This unique characteristic also plays a crucial role in fields like archaeology and palynology, as the exine is durable enough to fossilize in soil, preserving a record of its species over time. In essence, pollen grains serve as tiny, natural time capsules embedded in the earth**.(Figure 3)**

This outer layer is primarily composed of **s**poropollenin, an exceptionally resistant biopolymer. The surface and tiny crevices of the exine are coated and filled with a mostly lipid-based substance known as the pollen coat**.** This sticky coating plays a crucial role in pollination it helps pollen adhere to the bodies of pollinators and ensures the cohesion of the pollen pellets that bees carefully collect.

Interestingly, this layer is also equipped with tiny spines that latch onto the fine hairs of bees, making pollen transfer even more efficient. Despite the presence of wax on its surface,

bees have developed the ability to digest it, transforming pollen into **bee bread** or **royal jelly**, which serves as nourishment for young larvae **(Thibault, 2017).**

- **The Intine:** The Delicate Inner Layer

The intine is the thin and fragile inner layer of the pollen grain, yet it plays a crucial role in determining its properties. This layer is composed of gel-like fatty substances rich in carotenoids, aromatic compounds, polyphenols, flavonoids, and fat-soluble antioxidant vitamins.

Made of pecto-cellulosic material, the intine surrounds the vegetative cell, which contains essential nutrient reserves needed for the growth of the pollen tube a key step in plant fertilization **(Thibault, 2017).**

## 3. Pollen Membrane and Its Structure

- **Apertures**

The surface of most pollen grains and spores features specific areas where the exine is less resistant, allowing for the emergence of the pollen tube or prothallus. These specialized regions are known as apertures **(Gastaldi, 2021).**

Sometimes, around the apertures, the structure of the exine is altered. In such cases, these modifications are referred to as borders. These borders can be a characteristic of either the sexine or the nexine **(Moore et *al.*, 1991).** Different types of borders can be distinguished:

- **Annulus:** A thickening or thinning of the sexine around an ectoporus **(e.g., Avena sp.).**
- **Margo:** A region of the exine around an ectocolpus with distinct ornamentation or thickness

Different from the rest of the sexine **(e.g., Hedera sp.).**

- **Costa**: A thickening of the nexine around an endoaperture (e.g., Nyssa sp.) or beneath the edge of an ectoaperture.
- **Atrium or Vestibulum**: A space formed by the separation of diverging exine layers around a pore (e.g., Corylus sp.).
- **Oncus:** A lens-shaped structure located beneath an aperture (e.g., Corylus sp.).

- **Operculum**: A well-defined and isolated structure of the sexine/ectexine covering part of an ectoaperture (e.g., Dianthus sp.).

This classification highlights the structural diversity of pollen grains, which plays a crucial role in their identification and function.

➢ **Pollen Grain Release**

When pollen grains reach maturity, all the tissues of the anther undergo dehydration, causing a reduction in the surface area of the outer cell walls in the mechanical layer. The accumulated tension eventually leads to the dehiscence of the anther a process known as anthesis which typically occurs through two longitudinal slits. This allows the pollen grains to be released into the surrounding environment **(Marouf, 2007).**

## 4. Pollination

There are two main types of pollination: self-pollination and cross-pollination. Pollination occurs when the ovules of a flower germinate and fertilize, enabling the production of seeds and fruits.

Various agents known as pollination vectors carry out pollen transport. These vectors can be:

- Biotic, such as birds, insects (mainly bees), bats, and other animals.
- Abiotic, such as wind or water.

- **List of Biotic Pollination Types**

Biotic pollination occurs through various mechanisms, each with a specific name based on the mode of pollination or the type of organism involved. The main types of pollination are:

- **Anemophily:** Pollination by wind, occurring in all gymnosperms and about 20% of angiosperm species. Most trees and grasses in temperate regions rely on wind for pollination **(Reece et *al.,* 2012).** Anemophilous pollen is the primary cause of pollen allergies in temperate regions.
- **Hydrophily:** Pollination by water.
- **Ornithophily:** Pollination by birds.
- **Chiropterophily:** Pollination by bats.

- **Entomophily:** Pollination by insects, with approximately 65% of flowering plants mdepending on insects for pollination. Bees are the most important pollinators, though butterflies and flies also contribute **(Reece et *al.*, 2012).**

## 5. Chemical Composition of Pollen

The global chemical analysis of pollen helps determine its composition **(Pons, 1970).** The main components of bee pollen include:

- Carbohydrates (13–55%).
- Fibers (0.3–20%).
- Proteins (10–40%).
- Lipids (1–10%).

Other important constituents include minerals, vitamins, carotenoids, phenols, flavonoids, and phytosterols **(Feás et *al.*, 2012).** The following table presents the average percentages of key elements found in pollen grains **(Table 1).**

**Table 1:** Chemical Composition of Pollen in Percentage (Relative to Dry Weight) (Pons, 1970).

| **Principaux constituants** | **Pourcentage (%)** |
|---|---|
| .<br><br><br>Glucides (sources) Lipides<br>(corps gras) Protides<br>Les protéines allergéniques L'antigène E<br>L'antigène K | 8 à 16<br>3 à 5<br><br>25 à 42 1<br>à 20<br>11 à 29<br>0.5 à 1<br>0.5 – 6<br>3 |
| Sels minéraux | 1 à 8 |
| Cendres | 5 |
| Corps indéterminés<br>(substances antibiotiques actives…) | 20 |
| Rutine | 0.017 |
| Pigments | Traces |
| Un grand nombre de vitamines (B1 jusqu'à B12, C, D, E, H) | 0.015 |
| Flavonoïdes, slavonnes, diclicorsides stérols marindinueapiginine | Traces |

## 6. Properties and Uses of Pollen

Pollen grains are the primary source of protein essential for the survival of bees. During their foraging flights, worker bees collect pollen grains from flowers and transport them as small pellets attached to their hind legs. Once gathered, the pollen is stored inside the hive in specific honeycomb cells, separate from those used for nectar storage. Additionally, beekeepers can collect bee pollen in pellet form using a pollen trap installed at the entrance of the hive **(Almeida-Muradian, 2005).**

### ➢ Monofloral vs. Heterofloral Pollen

Bee pollen pellets can be either monofloral or heterofloral:

- If foraging bees find a sufficient supply from a single plant species, the pollen remains monofloral, preserving the organoleptic and biochemical properties of its original plant source.

- If the pollen supply is insufficient, bees will visit multiple plant species, sometimes mixing different pollen types within a single pellet. Heterofloral pollen has a more diverse composition and properties **(Stanley & Linskens, 1974).**

From a biochemical perspective, bee pollen is not only a rich protein source but also contains:

- Carbohydrates, lipids, minerals, vitamins, water, and trace elements **(Tomas-Lorente et *al.*, 1992).**
- Lipid-based pigments from flower anthers, which influence its color. Depending on its floral origin, pollen pellets can range from white and cream to dark brown, with yellow, orange, red, green, and gray being the most common colors **(Stanley & Linskens, 1974).**

### ➢ Health Benefits of Pollen

- Pollen can be used as a daily supplement to address nutritional deficiencies. Studies on animals suggest its benefits in:
- Reproduction, growth, and digestion, helping regulate both constipation and diarrhea.
- Antibiotic properties, particularly against Salmonella.
- Boosting energy levels, making it useful for mental and physical fatigue.

- Supporting liver function, managing hypertension, and addressing prostate issues **(El Hady et *al.*, 1986).**

Thanks to its diverse bioactive components, pollen holds significant potential in various fields, including nutrition, medicine, and natural health.

## II. Palynology

### 1. Definition

Palynology is the study of pollen grains and spores, whether modern or fossilized. When dealing with fossilized specimens, it is referred to as paleopalynology **(Hyde & Williams, 1944).**

The term "palynology" (from the Greek: to scatter, to sprinkle, derived from the word for flour or pollen dust) is now universally used to describe all scientific research focused on pollen grains and spores, either as subjects of study or as investigative tools **(Pons, 1970).**

### 2. Applications of Palynology

Palynology is a valuable tool with various applications, including the study of pollen and spore production and dispersion in the atmosphere (aerobiology), research on human allergies, archaeological excavations of shipwrecks, and detailed analysis of animal diets **(Jarzen & Nichols, 1996).**

Palynology is divided into two main branches: fundamental palynology and applied palynology. The primary application of fundamental palynology is plant classification. Meanwhile, applied palynology is utilized in several fields, including

- **Mellissopalynology**

Mellissopalynology is the study of pollen in honey to identify the plant sources used by bees in honey production. This analysis is crucial for beekeepers, as honey derived from specific plants such as mesquite, buckwheat, and citrus often commands a higher market price than honey from other floral sources. Additionally, some plants produce nectar and pollen that can be harmful to human health. By monitoring the types of pollen present in honey, it is possible to detect toxic sources, ensuring that potentially harmful honey is excluded from commercial distribution **(Jarzen & McAllister, 1994).**

Mellissopalynology is widely used to assess the purity, geographical origin, and floral composition of honey. Furthermore, it helps analyze correlations with environmental factors such as precipitation and temperature, which influence pollinators and pollination networks **(Louveaux, 1970; Nascimento, 2014).**

- **Paleopalynology**

Paleopalynology is the study of organic microfossils found in macerated sedimentary rock samples. These microfossils consist, at least partially, of highly resistant organic molecules, usually sporopollenin, chitin, or "pseudochitin," with some exceptions. Palynomorphs are commonly within the size range of approximately 5–500 µm (micrometers). However, some megaspores are significantly larger, particularly late Paleozoic "seed" megaspores, which can reach considerable dimensions. According to Potonie and Kremp (1955), species of Tuberculatisporites can measure up to 3000 µm (3 mm), while megaspores of Cystosporites are even larger, reaching up to 3 × 8 mm **(Schopf, 1938).**

Unlike other plant structures, the exine of pollen grains is well-preserved in sediments under anoxic conditions due to its sporopollenin composition. This highly durable material can withstand geological time periods without significant alteration **(Cerceau et *al.*, 1993).**

- **Pharmacopalynology**

Pollen is considered one of the most nutrient-rich foods, containing vitamins, essential amino acids, proteins, fatty acids, minerals, enzymes, and other bioactive compounds such as flavonoids **(Donadieu, 1983).** Various therapeutic properties of pollen have been identified by **Langler (2002).** It helps regulate functional disorders by balancing intestinal flora and positively influences the hormonal system by reducing stress and premature aging.

Due to its flavonoid content, pollen supports blood circulation by strengthening veins and arteries. Additionally, it enhances cellular metabolism thanks to its diverse composition of amino acids, trace elements, and vitamins, which are essential for protein synthesis. Regular consumption of pollen has been shown to improve both physical and mental performance.

- **Biopalynology**

Pollen grains carry half of the chromosomes of higher plants, making them a valuable genetic resource for plant breeding and improvement programs. However, once released into the atmosphere in their tricellular state, pollen naturally remains viable only for a few hours and, in some cases, a few days **(Cerceau, 1959).**

Long-term storage of bicellular pollen could enable the creation of pollen banks, such as the one established in 1983 at the Palynology Laboratory of the National Museum of Natural History in Paris **(Cauneau, 1988).**

Biopalynological events and structures can be utilized for various human applications. Moreover, they offer promising solutions to unresolved challenges in fields such as agro-food industries, horticulture, fruit tree cultivation, and even medicine **(Souvré et *al.*, 2014).**

- **Paleoclimatology**

In paleoclimatology (the study of past climates), applied palynology analyzes samples such as those obtained from core drilling to identify plant species that existed during specific periods. The information helps reconstruct past climates.

For example, the exclusive presence of Chenopodiaceae suggests an arid climate, whereas the coexistence of Chenopodiaceae and Artemisia indicates a semi-desert environment **(Rossignol, 1997).**

### 3. Pollen Morphology as a Phylogenetic Trait

Palynology is primarily based on the morphological characteristics of pollen, which serve as a crucial tool for identifying plant populations **(Jafari & Karimi, 2007; Quamar et *al.*, 2018).** Pollen consists of microscopic grains that act as the male microgametophytes, produced by the stamens (male reproductive organs) of flowering plants. Pollen carries a vast amount of genetic information and follows the evolutionary trends of plants, similar to other plant organs **(Perveen, 2000).**

Since pollen structure is less influenced by environmental factors, it exhibits strong genetic conservatism. Traits such as shape, size, polarity, surface ornamentation, and the number and type of germination structures remain relatively stable, often reflecting shared characteristics within a plant family or genus **(Bahadur et *al.*, 2018; Kailas et *al.*, 2016).** Examining pollen morphology helps determine plant origins and evolutionary relationships, serving as a critical foundation for botanical, archaeological, and geological research. Additionally, pollen is a seasonal allergen **(Devender et *al.*, 2016),** and studying its structure provides essential insights for immunological analysis.

## *III. Spartieum junceum L*

### 1. Generality of Spanish Broom *(Spartieum junceum L)*

Spanish Broom (*Spartieum junceum L.*) is native to Europe, North Africa, and Western Asia. It belongs to the legume family, specifically the subfamily of papilionaceous plants. In this group, the flower's corolla is distinctly irregular. The two lower petals are fused into a single structure called the keel, which encloses the stamens and the pistil. This keel is partially covered by the lateral petals, known as wings, which in turn are topped by a much larger upper petal, referred to as the standard. The overall shape of the flower resembles a butterfly, hence the term papilionaceous, from the Latin papilio meaning butterfly.

The plant has ten stamens, whose filaments are fused together to form a sheath surrounding the ovary. This shrub can grow up to three meters tall. Its leaves appear in April during the flowering period and fall off in the summer, leaving behind bare stems that resemble rushes.

Known in English as Spanish broom, and in Algeria as Tertak, its Latin name *Spartieum junceum L* reflects both its traditional uses and physical characteristics Spartieum junceum L comes from the Greek spartos, meaning "cord" or "rope," referencing the historical use of its stems. The species name junceum alludes to rushes (juncus in Latin), due to the cylindrical shape of its stems, which resemble those of the rush plant. **(Devender et *al.*, 2016).**



**Figure 4 :** *Spartieum junceum L*

## 1. Botanical description

Spanish broom is a rapidly growing, deciduous shrub, 10 to 15 ft tall. The stems are long, smooth, slender, cylindrical and erect with few branches, appearing rush-like. Spanish broom leaves are small, 0.5 to 1 inch long, oval, and smooth-margined. Leaves are ephemeral, remaining on the plant for 4 months or less, giving the plant a leafless appearance. Because the leaves are small and deciduous, the stems are green and are the primary photosynthetic tissue of the plant**.( Di Tomaso et *al.*, 2013).**

The yellow, pes-like flowers are produced on current-year shoots in terminal racemes with several flowers The lowers are large, up to 1 inch long, and grow on short stalks on both sides of the main stem. Reproduction  sby seed and plants begin flowering from 18 months to 3 years of age. Seeds are produced in slightly flattened pods 1.5 to 4 inches long, When mature, pods are dark brown, covered with long, silky, silvery hairs, and contain 30 se 18 sonds Seed depenal occurs when pods eject the seeds several feet from the plant Seeds cam semain vathie the soil for up to 30 years. Large soil seedbanks often accumulate making kang m Alllical Susan may live for sup to 30 years**.( Di Tomaso et *al.*, 2013).**



**Figure 5 :** Floral of Spanish Broom *Spartieum junceum*

**2. Classification**

| | |
|---|---|
| **Kingdom** | Plantae |
| **Clade** | Tracheophytes |
| **Clade** | Angiosperms |
| **Clade** | Eudicots |
| **Clade** | Rosids |
| **Order** | Fabales |
| **Family:** | Fabaceae (Leguminosae) – the legume or pea family |
| **Subfamily** | Faboideae (Papilionoideae) – the papilionaceous legumes |
| **Genus** | Spartium |
| **Species** | *Spartieum junceum L* |

**3. Uses of Spanish Broom Spartieum junceum L**

- **Fiber Production**

The long, flexible stems of *Spartieum junceum L* have traditionally been used to make ropes, cords, mats, and textiles. The bast fibers extracted from the stems are strong and were once important in rural and coastal Mediterranean communities. **(Degiovanni et *al.*,2003).**

- **Ornamental Use**

Due to its bright yellow, fragrant flowers and drought resistance, Spanish Broom is commonly used as an ornamental shrub in gardens and landscaping, especially in arid regions**.( Gilman& Watson, 1994).**

- **Soil Stabilization & Erosion Control**

Its deep root system helps prevent soil erosion, making it useful for stabilizing slopes, dunes, and degraded land in Mediterranean areas**.( Pignatti, 1982).**

- **Traditional Medicine**

In folk medicine, parts of the plant (especially flowers and seeds) have been used for their diuretic, purgative, and emetic properties. However, caution is needed due to the plant's toxicity**.( Duke, 2002).**

- **Perfume & Fragrance Industry**

The flowers are fragrant and have been used in the extraction of essential oils for perfumes, although not as commonly today**.( Bown, 2001).**

- **Ecological & Biodiversity Value**

The plant provides shelter and nectar for pollinators and small fauna. It plays a role in the biodiversity of Mediterranean shrublands**.( Quézel & Médail ,2003).**

# Materials and Methods

## *Materials and Methods*

### 2. Materials and Methods

#### 1. Plant material

The plant material used in this study consists of flowers collected from wild-growing plants of (*Spartieum junceum L*.), belonging to Fabaceae family. These samples were gathered from Tebessa region.

#### 2. Study area

Tebessa department covers an area of 14,227 km² and is situated at an altitude of 960 meters. It is naturally connected to the vast steppe region of the country. Located within a semi-arid bioclimatic zone of North Africa, it lies between two mountain ranges of the Atlas: the Tellian range in the north and the Saharan range in the south.

A significant climatic gradient exists from north to south across this region, with more or less mesic conditions in the north and hot hyper-arid conditions in the south **(Mekahlia et *al.*, 2013).**

#### 3. Sampling

A random sampling was carried out regarding the *Spartieum junceum L* plants .A set of more than 50 pollen grains was selected from the anthers of the flowers of the studied accessions.

#### 4. Morphological Characterization:

In order to determine the morphological features of the pollen grains, we employed a standardized morphological identification key. The analysis was based primarily on the classification system and descriptive criteria proposed by Erdtman (1952), a foundational reference in palynology. This approach allowed for the detailed examination of pollen shape, size, aperture type, and surface ornamentation under light microscopy. By applying Erdtman's methodology, we ensured consistency and scientific validity in the identification process. For clarity and reproducibility.

#### 5. Visualization

Initially, the collected flowers were examined using a binocular loupe at magnifications that allowed for a clear and detailed view, suitable for study. The selected pollen grains were then placed on a slide for identification, and observation was carried out using an optical microscope at 40x magnification. Digital photographs were taken and subsequently processed using Microsoft Office Picture Manager.

## *Materials and Methods*

As previously mentioned, we followed the method outlined by Erdtman, 1952, who identified eight shape classes based on the ratio of the polar axis (P) to the equatorial diameter (E). The P/E ratio, multiplied by 100, provides a precise characterization of the pollen shape. Accordingly, the pollen types were classified as shown in Table 2.

**Table 2 :**Pollen shape classes and suggested relationship between the polar axis (P) and the equatorial diameter (E) (Erdtman, 1952).

| Pollen Shape Classes | P/E | 100 × P/E |
|---|---|---|
| **Peroblate** | <5/10 | <70 |
| **Oblate** | 5/10 -7/10 | 60 -95 |
| **Suboblate** | 6/10 -7/10 | 75 -90 |
| **Spheroidal Oblate** | 8/10 -10/10 | 90 -100 |
| **Spheroidal Prolate** | 10/10 -10/7 | 100 -114 |
| **Subprolate** | 10/7-10/6 | 114 -133 |
| **Prolate** | 10/7 -10/5 | 133 -200 |
| **Perprolate** | >10/5 | >200 |

### 6. Statistical analysis

All statistical analyses and data processing were performed using Minitab 2015. The objective was to characterize morphological variation among pollen grains, identify potential clusters or sub-populations, and evaluate the extent of diversity within and between these groups.

# Résultats and discussions

## 3. Resultats

### 1. Dendrogram construction

The analysis of the dendrogram **(Figure7)** reveals that it consists of five groups, by drawing a horizontal line at appproximately 66.67% similarity, the dendrogram is divided into five distinct clusters.

The first one is composed of accessions 3 and 8 at the similarity level of threshold of 33.33%, on the other hand, accessions 6 , 10, 13 , 22 and 14 , 16 , 17, 20 , 25 constitute the second group with a level of similarity of 100% , moreover accessions 2,7,11,19,21,23,28 are also 100% similar and form the thrid group.

Plus, the fourth groupe which is made up of three subgroups, where the first one is composed by accessions 15,18,26,29 and 24, the second one is composed only by accession 9 , while the third one by accessions 1,5,12, 27,3.

Finally , accession 4 which corresponds to the last group joins the other clusters at a similarity threshold of 0%.

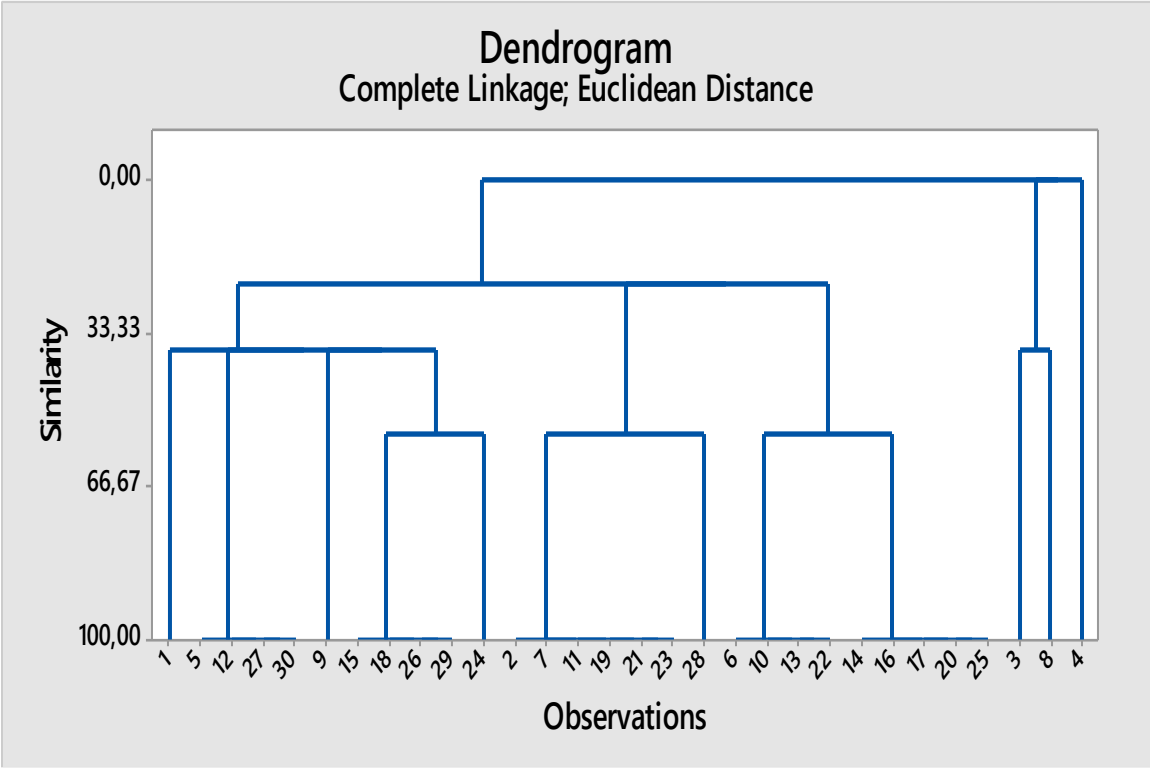



**Figure 7:** Dendrogram illustrating the clustering of the studied accessions

## *Résultats and discussion*

### 4. Discussion of results

The present study highlights a remarkable morphological diversity among pollen grains of *Spartieum junceum L* It is noted that pollen diversity greatly contributes to the enrichment of palynological databases and thus helps in understanding the mechanisms of taxonomic speciation."

### 1. Pollen grain patterns observed

He most common pollen types in *Spartieum junceum L* are spheroidal prolate and subprolate. Furthermore, the presence of other forms such as oblate, suboblate, and spheroidal oblate was also recorded."

These findings confirm the presence of both intra- and interplant polymorphism. In contrast, some forms such as perprolate and peroblate are rare or even absent in the majority of accessions.

### 2. Factors influencing pollen grain shape

Regarding the intraspecific variation in pollen size, several studies have addressed this issue in an attempt to explain these evolutionary changes **(Cruden, 2009).** For their part, Beaulieu et al. (2008) suggested that ploidy and/or genome size could be predictors that help explain the size factor of certain pollen grains.

Furthermore, pollen size may vary in response to a range of environmental and biotic conditions **(Torres, 2000),** including air temperature and humidity **(Firon et *al.*, 2012)**

On the other hand, Katifori and Hall (2010) stated that in dry environments, the pollen wall has the ability to fold in on itself in order to conserve water and prevent dehydration. Furthermore, plants coexisting in the same habitat with limited resources and/or living under harsh conditions may produce pollen with an affected phenotype **(Snow and Mazer, 1988).**

Other factors can influence pollen grain phenotypes. Among these factors is altitude **(Prieu, 2015)**.

### 3. Genetic Aspects and Phenotypic Plasticity

In natural populations, genetic variation can arise from several sources, including mutations, gene flow between populations, and environmental heterogeneity within populations **(Schlichting et *al.*, 1990).** These genetic differences provide the raw material for adaptation and evolution by natural selection.

Phenotypic plasticity the capacity of a single genotype to produce different phenotypes depending on environmental conditions is also fundamental for plant survival and reproduction. It allows individuals to adjust traits such as pollen grain morphology, flowering time, or drought tolerance in response to fluctuating environments **(Bradshaw, 1965; Sultan, 2000).**

For example, Bradshaw (1965) demonstrated that plasticity in plant traits can buffer populations against environmental variability, while Sultan (2000) highlighted the importance of plastic responses in promoting ecological success and potential speciation.

The interaction between genetic variation and phenotypic plasticity shapes population diversity and evolutionary potential, influencing processes like speciation and adaptation to new environments **(Pigliucci, 2001).**

Moreover, some variations may correspond to phenotypic plasticity, which can be defined as the change of a trait in response to environmental variation for a given genotype **(Lefèvre et *al.*, 2015).**

Pollen diversity greatly contributes to the enrichment of pollen databases and thus helps in understanding the mechanisms of taxonomic and functional speciation.

**4. Factors Influencing Pollen Grain Shape**

Regarding intraspecific variation in pollen size, several studies have addressed this question with various hypotheses to explain these evolutionary changes **(Ejsmond et *al.*, 2011; Cruden, 2009**). For their part, Beaulieu et al. (2008) suggested that polyploidy and/or genome size may be predictors that help explain the larger size of certain pollen grains.

In fact, pollen grain size can vary depending on a range of environmental and biotic variables. Growth conditions must therefore be controlled **(Stephenson et *al.*, 1994; Torres, 2000).** Among these factors, air temperature and humidity are the most critical **(Firon et *al.*, 2012).**

Moreover, according to Firon et *al.* (2012) and Garcia et *al.* (2017), abiotic stresses affect pollen development before anther dehiscence. Pollen grains undergo significant dehydration, typically losing about 15 to 35% of their cytoplasmic water. Furthermore, under certain conditions, this water loss leads to a reduction in pollen grain volume, and the pollen wall folds along the apertures **(Nepi et *al.*, 2001).**

Furthermore, plants coexisting in the same habitat with limited resources or under extreme conditions compete for the resources necessary for their growth. This competition also affects pollen, where it can influence the pollen phenotype of the offspring **(Snow & Mazer, 1988).**

Other factors can lead to phenotypic changes in pollen. Among these, altitude plays an important role in the morphological diversity of pollen. According to Prieu (2015), four-aperture pollen grains are more frequent at high altitudes, while five-aperture grains dominate at lower elevations. Moreover, in certain plant species, elevation affects pollination efficiency, which tends to decrease with increasing altitude **(Till-Bottraud, 1999).**

# Conclusion

## *Conclusion*

This study of palynological polymorphism in a plant species native to the northern Tebessa region, *Spartieum junceum L*, has yielded significant insights into the morphological variability of pollen grains.

A wide range of pollen forms was identified among the studied accessions. Some morphotypes, such as spheroidal prolate, suboblate and prolate forms are more common, while other forms, like peroblate, oblate and perprolate were very sporadic. In contrast, certain forms described by Erdtman (1953), such as the suboblate were not recorded in the studied species.

The observed morphological diversity is primarily attributed to abiotic factors, particularly air temperature, which affects both the size and shape of pollen grains **(Erdtman ;1969 , Moore et *al.*, 1991).** In addition, genetic mutations may also contribute to this variability, as highlighted in studies on developmental plasticity and morphological evolution **(West-Eberhard, 2003).**

Statistical analyses, including cluster analysis and dendrogram construction, confirmed the presence of notable polymorphism among the samples, indicating a complex pattern of morphological differentiation.

To further elucidate the causes of palynological variability, future research should include a larger number of accessions from different ecological zones within Tebessa and across other regions of Algeria. Moreover, integrating genomic studies would be a valuable step toward a better understanding of pollen polymorphism, the standardization of identification keys, and the enrichment of palynological databases **(Andersen, 2006; Punt et *al.*, 2007).**

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# Appendix

**Appendix 01 :**

| Shape / Flower | **Peroblate** | **Oblate** | **Suboblate** | **Spheroidal oblate** | **Spheroidal prolate** | **subprolate** | **prolate** | **perprolate** |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 4 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 9 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 16 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 23 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| 24 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 27 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |

**Appendix 02 :**

