



## Some anatomical and palynological characteristics of *Onobrychis paucijuga* Bornm. (Fabaceae)

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### Abstract

**Purpose:** The present study aims to provide some anatomical and palynological characterization of the endemic species *Onobrychis paucijuga* Bornm. and to assess its taxonomic significance within the genus.

**Method:** Root and stem anatomical structures were examined using light microscopy. Hand sections obtained from plant materials preserved in 70% ethanol were mounted in glycerin–gelatin and analyzed under a Nikon Eclipse 80i microscope. Pollen morphology was investigated using both Wodehouse and Erdtman methods. In addition, scanning electron microscopy (SEM) was employed to examine exine ornamentation and surface ultrastructure.

**Findings:** The root exhibits a well-developed secondary structure with a multilayered periderm, a wide cortex composed of parenchymatous cells, and a distinct vascular cambium separating the secondary phloem and xylem. Sclerenchymatous fibers occur in the cortical region and near the phloem. The stem shows a typical dicotyledonous structure with a collenchymatous hypodermis, multilayered lacunar collenchyma, and well-developed vascular tissues. In older stems, pith parenchyma cells tend to disintegrate. Pollen grains are radially symmetrical, isopolar, and tricolpate. Based on the P/E ratio, they are prolate in the Wodehouse preparation and subprolate in the Erdtman preparation. The exine ornamentation is reticulate-granulate, and the exine is tectate, with a thin tectum. SEM observations confirmed the reticulate-granulate exine ornamentation and revealed detailed surface structures.

**Conclusion:** The anatomical and palynological features of *O. paucijuga* are largely consistent with those reported for other members of the genus *Onobrychis* and the family Fabaceae. The combination of anatomical and pollen morphological characters provides valuable diagnostic traits for the identification and taxonomic evaluation of this endemic species.

**Keywords:** Fabaceae, *Onobrychis paucijuga*, anatomy, palynology, pollen morphology, SEM

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### *Onobrychis paucijuga* Bornm. (Fabaceae)'nın bazı anatomik ve palinolojik özellikleri

#### Özet

**Amaç:** Bu çalışma, endemik tür *Onobrychis paucijuga* Bornm. (Fabaceae)'nin bazı anatomik ve palinolojik özelliklerini ortaya koymayı ve bu özelliklerin cins içerisindeki taksonomik önemini değerlendirmeyi amaçlamaktadır.

**Metod:** Kök ve gövde anatomik yapıları ışık mikroskobu kullanılarak incelenmiştir. %70 etanolde muhafaza edilen bitki materyallerinden elde edilen el kesitleri gliserin-jelatin ile preparat haline getirilmiş ve Nikon Eclipse 80i mikroskobunda analiz edilmiştir. Polen morfolojisi Wodehouse ve Erdtman yöntemleri kullanılarak incelenmiştir. Ayrıca, ekzin ornamentasyonu ve yüzey ultrastrüktürünü belirlemek amacıyla taramalı elektron mikroskobu (SEM) kullanılmıştır.

**Bulgular:** Kök, çok katlı periderm, parenkimatik hücrelerden oluşan geniş bir korteks ve sekonder floem ile ksilemi ayıran belirgin bir vasküler kambium ile karakterize edilen iyi gelişmiş bir sekonder yapıya sahiptir. Korteks bölgesinde ve floeme yakın alanlarda sklerenkimatik lifler gözlenmiştir. Gövde, kollenkimatik hipodermis, çok katlı

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laküner kollenkima ve iyi gelişmiş iletim dokularını içeren tipik bir dikotil yapı göstermektedir. Yaşlı gövdelerde öz parenkima hücrelerinin parçalanma eğiliminde olduğu belirlenmiştir. Polen taneleri radyal simetrik, izopolar ve trikolpattır. P/E oranına göre, Wodehouse preparasyonunda prolat, Erdtman preparasyonunda ise subprolat şekil göstermektedir. Ekzin ornamentasyonu retikülat-granülât olup, ekzin ince bir tektuma sahip tektat yapıdadır. SEM gözlemleri, retikülat-granülât ekzin ornamentasyonunu doğrulamış ve yüzey yapılarına ilişkin ayrıntılı bilgiler ortaya koymuştur.

**Sonuç:** *O. paucijuga*'nın anatomik ve palinolojik özellikleri, *Onobrychis* cinsinin diğer üyeleri ve Fabaceae familyası için bildirilen genel özelliklerle büyük ölçüde uyumludur. Anatomik ve polen morfolojik karakterlerin birlikte değerlendirilmesi, bu endemik türün tanımlanması ve taksonomik değerlendirilmesi açısından önemli ayırt edici özellikler sunmaktadır.

**Anahtar kelimeler:** Fabaceae, *Onobrychis paucijuga*, anatomi, palinoloji, polen morfolojisi, SEM

## 1. Introduction

Family Fabaceae (Leguminosae) is one of the largest and most diverse families of flowering plants, comprising approximately 770 genera and more than 19.500 species distributed worldwide [1]. Members of this family play a significant role in both natural ecosystems and agricultural systems due to their ecological adaptability and economic importance [1; 2]. Many Fabaceae species contribute to soil fertility through their ability to establish symbiotic relationships with nitrogen-fixing bacteria [3]. This ecological function makes the family particularly important in maintaining soil productivity and supporting sustainable agriculture [2]. In addition to their ecological roles, many species within Fabaceae are widely used as forage crops, medicinal plants, and ornamental species. The family is also characterized by considerable morphological and anatomical diversity, which has attracted the attention of plant taxonomists and systematists for many years [4; 5].

The genus *Onobrychis* Mill., belonging to the tribe Hedysareae of the Fabaceae family, represents an important group of herbaceous perennial plants that are mainly distributed in temperate regions of Europe, Western Asia, and Central Asia [5; 6]. The genus includes approximately 200 species worldwide, many of which are adapted to dry and mountainous habitats [7]. Species of *Onobrychis* are particularly well known for their value as forage plants which are widely cultivated in many parts of the world. In addition to their agricultural importance, members of this genus exhibit remarkable morphological diversity, making them an interesting subject for taxonomic and systematic investigations. Turkey represents one of the important diversity centers for the genus, hosting 57 (66 taxa) *Onobrychis* species, including 35 endemic taxa restricted to specific geographic regions. The high level of endemism within the genus highlights the importance of detailed taxonomic and anatomical studies for understanding species diversity and evolutionary relationships [6; 8; 9; 10; 11].

Several palynological and anatomical studies have been conducted on various species of the genus *Onobrychis* in order to clarify their systematic relationships [12; 13; 14; 15; 16]. Previous research has demonstrated that pollen characters such as polar axis length, equatorial diameter, P/E ratio, aperture structure, and exine ornamentation may vary among species of the genus [17; 18]. Similarly, anatomical investigations focusing on vegetative organs have revealed distinctive structural features that may contribute to taxonomic differentiation [12; 16; 19; 20]. However, despite the increasing interest in anatomical and palynological studies within Fabaceae, comprehensive data for many endemic *Onobrychis* species remain limited. In particular, detailed investigations addressing both anatomical and palynological aspects of certain endemic taxa are still insufficient.

One such species is *Onobrychis paucijuga* Bornm., an endemic taxon that occurs in restricted habitats and represents part of the unique floristic diversity of its native region. Although the genus *Onobrychis* has been the subject of several systematic and morphological studies, information concerning the anatomical structure and pollen morphology of *O. paucijuga* is scarce. Detailed examination of these characters may provide valuable insights into the taxonomic position of the species and contribute to a better understanding of structural diversity within the genus. Moreover, anatomical and palynological data may help clarify relationships between closely related taxa and provide additional characters for species identification.

The present study aims to investigate the anatomical and palynological characteristics of the endemic species *Onobrychis paucijuga* (Fabaceae). In this context, detailed anatomical analyses of root and stem tissues were carried out using light microscopy. In addition, pollen grains were examined in order to determine their morphological features and diagnostic characters. The results obtained from these analyses are expected to contribute to the taxonomic evaluation of the species and to provide additional data for future systematic studies within the genus *Onobrychis*.

## 2. Materials and methods

The specimens used in this study were collected from natural habitats around Ballıhisar village, Sivrihisar district, Eskişehir Province, Türkiye, on 16 July 2024. The specimens were collected from different natural populations in the vicinity of Ballıhisar village. A total of ten mature specimens of *Onobrychis paucijuga* were collected. Of these,

four specimens were used for anatomical investigations, whereas the remaining six specimens were prepared as herbarium vouchers and deposited in the Herbarium of Eskişehir Osmangazi University, Department of Biology, Faculty of Science (ESOGU). For anatomical investigations, the roots and stems of selected specimens were fixed and preserved in 70% ethanol. Hand sections were obtained from the preserved materials and mounted in glycerin–gelatin in order to prepare permanent slides. The sections were examined and photographed using a Nikon Eclipse 80i light microscope. Relevant literature was used for tissue identification and for the comparative evaluation of anatomical characters [20; 21; 22].

For palynological analyses, pollen grains were obtained from mature flowers collected from 10–15 different individuals from various locations. During field collection, plant samples belonging to the taxon were placed separately in paper envelopes to prevent the mixing of pollen with different characteristics. Pollen morphology was examined using light microscopy. The exine layers were described according to the terminology proposed by Faegri and Iversen [23]. Pollen grains of *O. paucijuga* were prepared for light microscopic examination following the methods described by Wodehouse and Erdtman [24; 25]. Pollen grains were examined and measured under a light microscope using 10× and 40× objectives, whereas detailed observations were performed using a 100× plan oil-immersion objective. The thicknesses of the exine and intine were measured following the methods described by Wodehouse and Erdtman [24; 25]. For scanning electron microscopy (SEM) analysis, pollen grains were mounted on aluminum stubs using double-sided carbon adhesive tape and coated with gold using a sputter coater to enhance conductivity. The samples were examined with a Hitachi Regulus scanning electron microscope. Micrographs were obtained at suitable accelerating voltages to investigate exine ornamentation and surface ultrastructure in detail. Pollen morphological terminology was based on the works of Wodehouse, Erdtman, and Faegri and Iversen [23, 24, 25].

### 3. Results

#### 3.1. Root Anatomy

The transverse section of the root revealed a well-developed secondary structure. The outermost region was composed of a periderm consisting of 4–7 layers of cells. Beneath the periderm, a wide cortex region was observed, consisting of thin-walled parenchymatous cells with irregular, oval, or occasionally flattened rectangular shapes. In the inner part of the cortex, particularly near the outer region of the phloem, sclerenchymatous fiber cells were observed either singly or in small groups. The phloem was located external to the vascular cambium and was composed mainly of sieve elements and parenchymatous cells. A distinct vascular cambium layer separating the secondary phloem from the secondary xylem was clearly distinguishable. The xylem formed the largest portion of the vascular cylinder and contained numerous vessel elements with relatively large lumens. The secondary xylem, including vessel elements and xylem rays, occupied most of the vascular cylinder, whereas the primary xylem elements were located in the central region of the root (Figure 1).

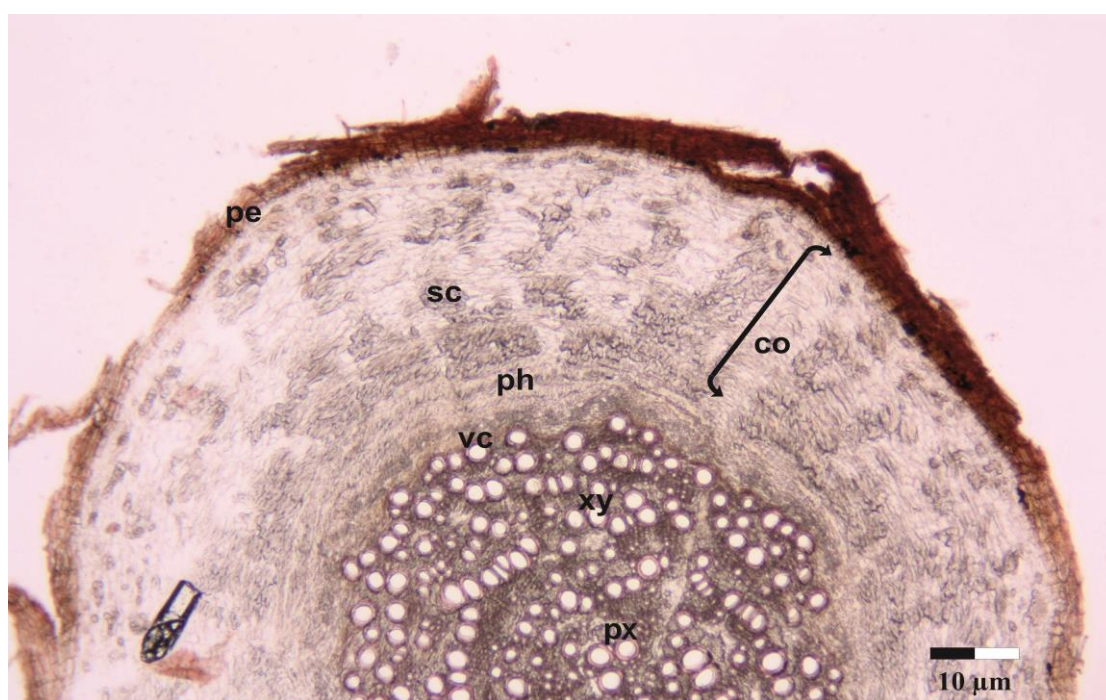


Figure 1. Transverse section of the root (**pe**, periderm; **co**, cortex; **sc**, sclerenchyma; **ph**, phloem; **vc**, vascular cambium; **xy**, xylem; **px**, primary xylem)

### 3.2. Stem Anatomy

The stem transverse sections of *Onobrychis paucijuga* were characterized by an irregularly undulate outline, with prominent ridges corresponding to vascular regions and shallow depressions between them. The epidermis consists of square, rectangular, or rectangular–oval cells and is sparsely covered with unicellular eglandular trichomes. Beneath the epidermis, a single layer of irregularly shaped collenchymatous hypodermis is found. Between the epidermis and the phloem sclerenchyma, multilayered lacunar collenchyma was observed in the cortex. The cortex is composed of parenchymatous cells that are oval or circular in shape. A sclerenchymatous bundle is present in the phloem region. The vascular cambium can be clearly distinguished between the phloem and xylem tissues. Secondary xylem vessels with tracheids occupying large area. Primary xylem represented by few vessels inwards. In the pith region, storage cells larger than the surrounding cells were observed. The pith consists of parenchymatous cells that are oval or circular in shape. In older stems, the pith parenchyma cells were observed to disintegrate (Figure 2).

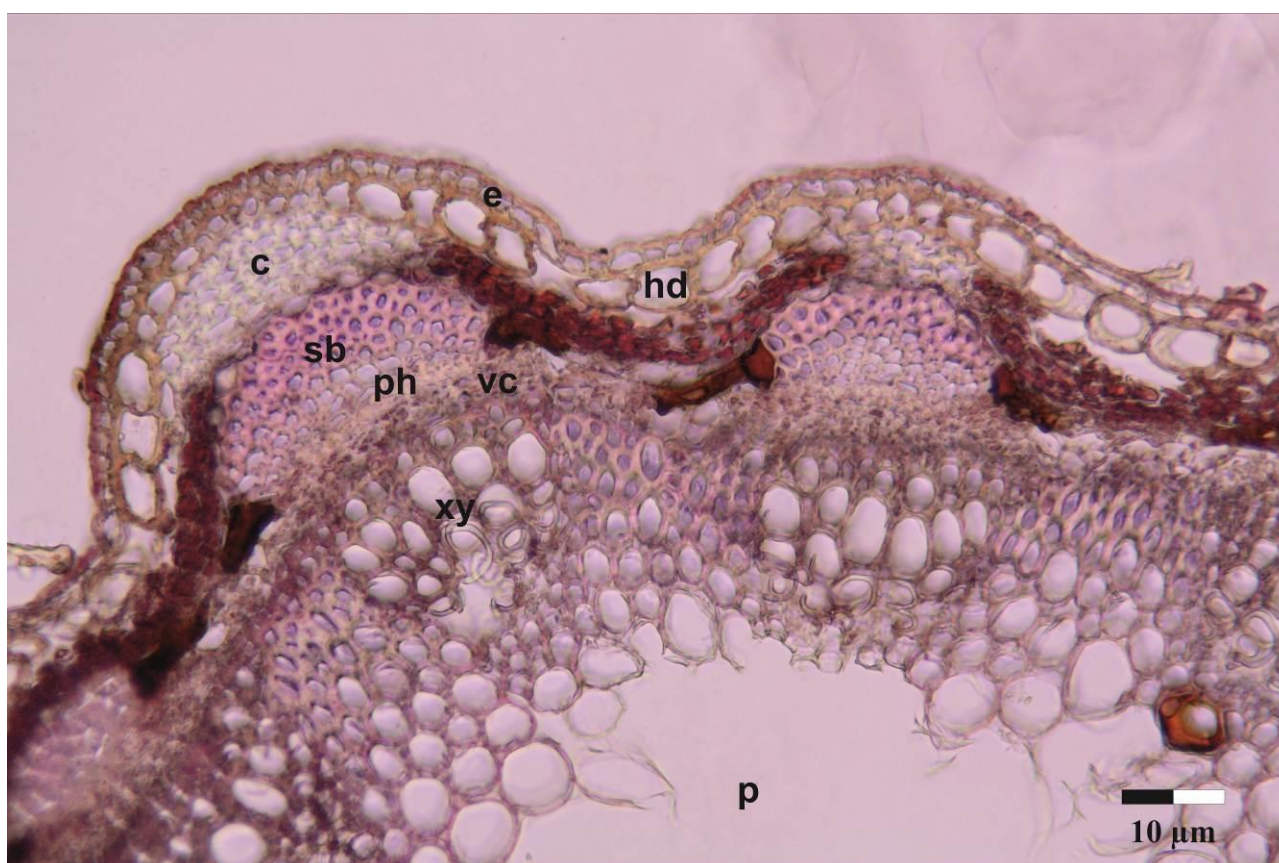


Figure 2. Transverse section of the stem (**e**, epidermis; **hd**, collenchymatous hypodermis; **c**, lacunar collenchyma; **sb**, sclerenchymatous bundle; **ph**, phloem; **vc**, vascular cambium; **xy**, xylem; **p**, pith)

### 3.3. Pollen Morphology

The pollen morphology of *Onobrychis paucijuga* was investigated using the Wodehouse and Erdtman methods (Figure 3A–D). The pollen grains are radially symmetrical, isopolar, and tricolpate. Based on the P/E ratio, the pollen grains are prolate in the non-acetolysed and subprolate in the acetolysed. In polar view, the pollen grains appear circular to subcircular, whereas in equatorial view they are elliptic. The polar axis measures 18.8  $\mu\text{m}$  (range 17–19  $\mu\text{m}$ ) and the equatorial axis measures 13.6  $\mu\text{m}$  (range 13–14  $\mu\text{m}$ ). The P/E ratio is 1.38 in the Wodehouse preparation and 1.21 in the Erdtman preparation, indicating prolate and subprolate pollen shapes, respectively. The pollen grains have tricolpate apertures; the colpi are long, narrow, and nearly syncolpate, with clearly defined smooth margins (Figure 3E). The apertures extend longitudinally along the polar axis and are distinctly visible under light microscopy. The exine ornamentation is reticulate-granulate and the tectum is thin and tectate in structure (Figure 3F). The average exine thickness is approximately 0.95  $\mu\text{m}$  in the Wodehouse preparation and 1.30  $\mu\text{m}$  in the Erdtman preparation. Overall, the pollen grains of *O. paucijuga* exhibit typical palynological characteristics observed in many members of the genus *Onobrychis* and the family Fabaceae.

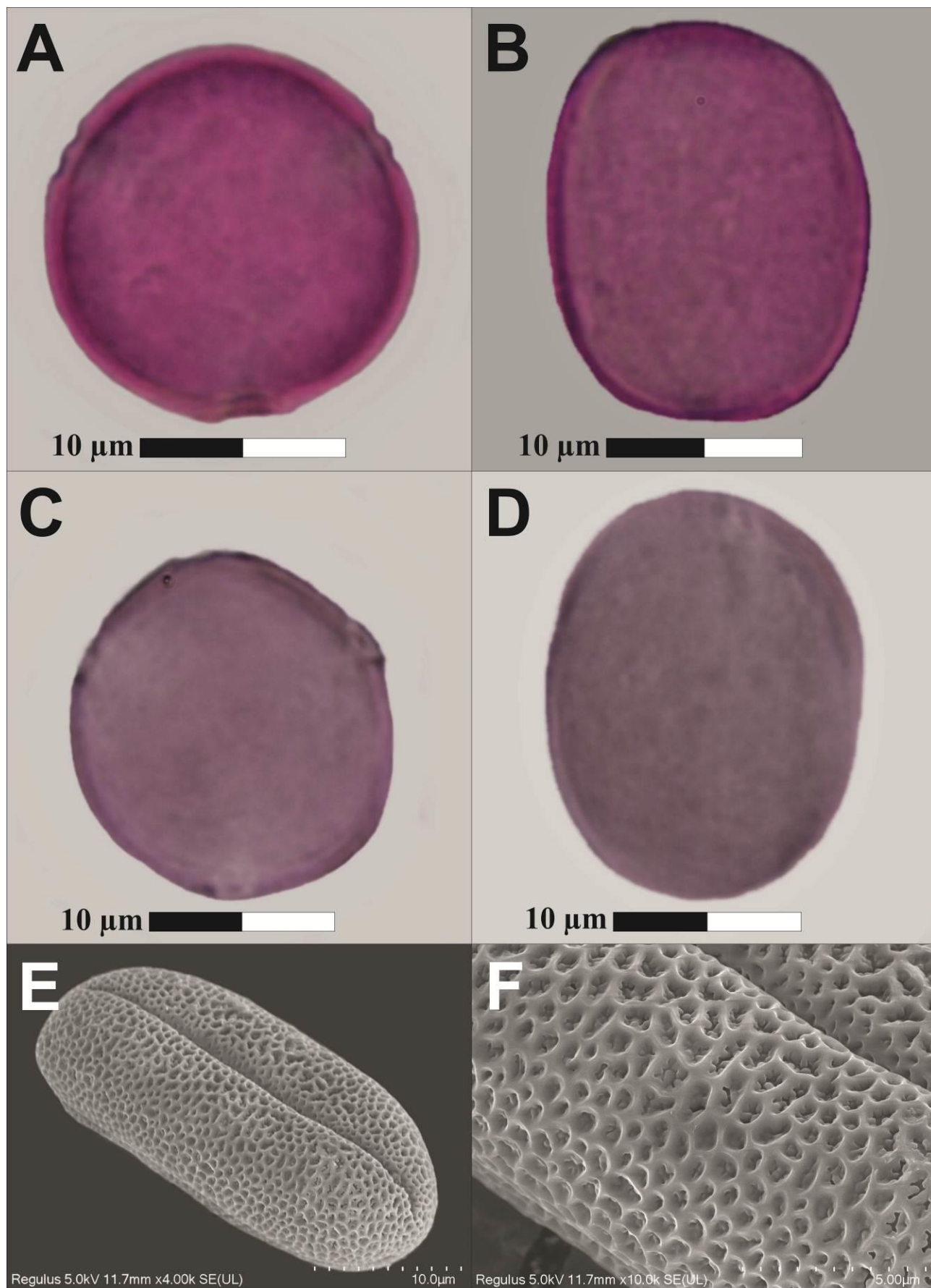


Figure 3. Pollen grains of *Onobrychis paucijuga* observed under light microscopy (A. Polar view (W); B. Equatorial view (W); C. Polar view (E); D. Equatorial view (E)) and SEM (E. General view; F. Exine ornamentation)

#### 4. Conclusions and discussion

The anatomical and palynological characteristics of *Onobrychis paucijuga* obtained in this study provide valuable data for understanding the structural features of this endemic species and contribute to the taxonomic knowledge of the genus *Onobrychis*. The anatomical structure observed in the root and stem sections of *O. paucijuga* largely corresponds with the general anatomical features reported for members of the genus and the family Fabaceae.

The root anatomy of *O. paucijuga* is characterized by a well-developed secondary structure with a multilayered periderm, a wide cortex composed of parenchymatous cells, and a clearly distinguishable vascular cambium separating the secondary phloem and secondary xylem. The presence of sclerenchymatous fibers within the cortical region and near the phloem was also observed. Similar anatomical features have been reported in other endemic *Onobrychis* taxa. In a comparative anatomical study of four endemic species from Turkey, the roots were also reported to possess multilayered periderm tissue, well-developed cortex regions composed of parenchymatous cells, and secondary vascular tissues formed by vascular cambium activity [16]. The presence of sclerenchymatous cells in the cortex or near the vascular tissues has been interpreted as a structural adaptation providing mechanical support in perennial herbaceous taxa of the genus. The similarity between the anatomical features observed in the present study and those reported by Tekin and Yılmaz suggests that these characters are relatively conserved within the genus [16].

The stem anatomy of *O. paucijuga* also displays typical dicotyledonous characteristics. The epidermis consists of rectangular to square cells and bears unicellular eglandular trichomes. Beneath the epidermis, a hypodermal collenchyma layer and multilayered lacunar collenchyma are present within the cortex region. The vascular tissues are arranged in a typical collateral structure, with phloem located external to the cambium and xylem forming a large portion of the vascular cylinder. Similar structural patterns have been described in previous anatomical studies on *Onobrychis* species. Tekin and Yılmaz reported that the stems of several endemic *Onobrychis* taxa possess epidermal layers with simple hairs, well-developed collenchyma in the cortex, and distinct vascular bundles accompanied by sclerenchymatous tissues [16]. Likewise, comparative anatomical investigations of perennial *Onobrychis* species from Iran indicated that variations in cortex thickness, vascular tissue arrangement, and pith structure may occur among species, although the general structural organization remains similar [12]. These observations support the idea that stem anatomical features can provide useful taxonomic characters at the species level within the genus.

In addition to root and stem anatomy, palynological characteristics constitute an important source of information for taxonomic and systematic studies in Fabaceae. The pollen grains of *O. paucijuga* were found to be radially symmetrical, isopolar, and tricolpate, with prolate shape in the Wodehouse preparation and subprolate shape in the Erdtman preparation. The exine ornamentation was reticulate-granulate. These findings are consistent with previous palynological studies conducted on different *Onobrychis* taxa. For example, Avcı et al. reported that the pollen grains of the genus *Onobrychis* are generally tricolpate, radially symmetrical, and prolate to subprolate in shape with reticulate or microreticulate exine ornamentation [17]. Similarly, studies carried out on Iranian taxa of the genus revealed that most species exhibit prolate pollen grains with reticulate or microreticulate ornamentation, indicating a high degree of similarity in pollen morphology across the genus [15].

The pollen size and aperture structure observed in *O. paucijuga* are also comparable with those reported in other regional studies. Pavlova and Manova demonstrated that pollen grains in *Onobrychis* species are typically medium-sized, tricolpate, and characterized by reticulate exine ornamentation, which represents a common pollen type within the tribe Hedysareae [14]. Similarly, quantitative analyses of pollen morphology in several *Onobrychis* taxa showed that polar and equatorial axes measurements may vary among species but generally fall within a similar range [18]. These results indicate that although pollen characters show limited variation, they still provide valuable information for distinguishing taxa when used together with other morphological and anatomical characters.

Despite the general similarity in pollen morphology among species of the genus, subtle differences in pollen size, exine thickness, and ornamentation patterns may contribute to species-level differentiation. Previous studies have suggested that pollen morphological characters in *Onobrychis* are relatively homogeneous but still possess diagnostic value when combined with other structural traits [15]. Furthermore, detailed palynological investigations of the genus have shown that the combination of pollen shape, aperture type, and exine sculpture plays an important role in clarifying taxonomic relationships among closely related taxa [17].

Recent morpho-anatomical and palynotaxonomic investigations on *Onobrychis* species have emphasized the systematic importance of combining anatomical and palynological data in taxonomic studies. Khan et al. reported that anatomical characteristics such as cortex structure, vascular tissue arrangement, and pith organization together with pollen features can significantly contribute to the delimitation of species within the genus [19]. The anatomical and palynological features observed in *O. paucijuga* in the present study are generally consistent with the patterns reported in these studies, supporting the reliability of these characters for systematic evaluations.

Overall, the results obtained from the anatomical and palynological analyses of *Onobrychis paucijuga* demonstrate that the species shares many structural features with other members of the genus while also exhibiting certain distinctive characteristics. The combination of root and stem anatomical traits with pollen morphological features provides useful diagnostic information for the identification and classification of this endemic taxon. These

findings contribute to the existing knowledge on the structural diversity of the genus *Onobrychis* and highlight the importance of integrated anatomical and palynological approaches in plant systematics.

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**Author contributions:** Okan Sezer designed the study, performed the research, collected and analyzed the data, and wrote the manuscript. The author read and approved the final version of the manuscript.

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