



SPORE MORPHOLOGY OF TWO *ASPLENIUM* L. (ASPLENIACEAE) SPECIES FROM TÜRKİYE

Türkiye'den İki *Asplenium* L. (Aspleniaceae) Türünün Spor Morfolojisi



Züleyha ASLAN ERGENEKON*

Arş.Gör.

Atatürk University,
Faculty of Science,
Department of Biology
ORCID: 0000-0002-5078-2317
azuleyha@atauni.edu.tr

Tülay EZER
Prof.Dr.

Niğde Ömer Halisdemir University,
Faculty of Architecture,
Department of Landscape
Architecture
ORCID: 0000-0002-6485-5505
tezer@ohu.edu.tr

*Sorumlu Yazar

Araştırma Makalesi

Geliş: 25.05.2025

Kabul: 12.07.2025

Anahtar Kelimeler

Asplenium trichomanes,
Asplenium ceterach, eğrelti

Keywords

Asplenium trichomanes,
Asplenium ceterach, fern

Bu çalışma, 11-13 Ekim 2023 tarihleri arasında Ankara'da düzenlenen "6th International Eurasian Conference on Biological and Chemical Sciences (EurasianBioChem 2023)" başlıklı kongrede sözlü özet bildiri olarak sunulmuştur.

Yazıların tüm teknik ve hukuki sorumluluğu yazarlarına aittir. İleri sürülen fikir ve iddialar Doğa ve Sürdürülebilirlik Derneğinin görüşünü yansıtmayabilir.

ABSTRACT

The genus *Asplenium* L. is represented by more than 750 fern species worldwide. In this study, it was aimed to contribute to the taxonomic evaluation of *Asplenium ceterach* L. and *Asplenium trichomanes* L. among 20 *Asplenium* species naturally distributed in Türkiye by examining their spore morphology. The specimens of the species examined in this study were collected from their natural distribution areas in Türkiye. Spore preparations were prepared by the acetolysis method, and morphological characteristics were analyzed in detail through light microscopy (LM) and scanning electron microscopy (SEM). As a result of the examinations, it was determined that the spore shape of both species was oblate. *A. ceterach* is monolet-dominant but rarely has trilete spores and exhibits a heteromorphic character. The aperture length of monolet spores varies between 33.00-45.00 μm . The mean polar axis length was 35.46 μm , and the equatorial axis length was 47.60 μm in spores of this species classified as medium-sized. *A. trichomanes* has only monolet spores. The aperture length of this species varies between 25.00-35.00 μm , with a mean polar axis of 28.89 μm and a mean equatorial axis of 41.91 μm . The thickness of the exine of both species is similar and varies between 1.00-2.00 μm . The findings suggest that the spore morphology of the studied species provides distinctive characters and can be considered as a complementary criterion in taxonomic classification.

ÖZET

Asplenium L. cinsi, dünya genelinde 750'den fazla eğrelti türü ile temsil edilmektedir. Bu çalışmada, Türkiye'de doğal olarak yayılış gösteren 20 *Asplenium* türünden *Asplenium ceterach* L. ve *Asplenium trichomanes* L. türlerinin spor morfolojileri incelenerek taksonomik değerlendirmelerine katkı sağlanması amaçlanmıştır. Çalışmada incelenen türlerin örnekleri, Türkiye'de doğal yayılış alanlarından toplanmıştır. Spor preparatları asetoliz yöntemiyle hazırlanmış ve morfolojik özellikler ışık mikroskobu (LM) ve taramalı elektron mikroskobu (SEM) ile ayrıntılı olarak analiz edilmiştir. İncelemeler sonucunda her iki türün de spor şeklinin oblat olduğu belirlenmiştir. *A. ceterach*, monolet baskın olmakla birlikte nadiren trilet sporlara da sahip olup heteromorf bir karakter sergilemektedir. Monolet sporlarda apertür uzunluğu 33.00-45.00 μm arasında değişmektedir. Orta büyüklükte sınıflandırılan bu türün sporlarında ortalama polar eksen uzunluğu 35,46 μm , ekvatorial eksen uzunluğu ise 47,60 μm olarak ölçülmüştür. *A. trichomanes* yalnızca monolet sporlara sahiptir. Bu türde apertür uzunluğu 25-35 μm aralığında değişmekte, polar eksen ortalaması 28.89 μm , ekvatorial eksen ise ortalama 41,91 μm olarak belirlenmiştir. Her iki türün ekzin kalınlıkları benzerlik göstermekte olup 1.00-2.00 μm arasında değişiklik göstermektedir. Elde edilen bulgular, incelenen türlerin spor morfolojilerinin ayırt edici karakterler sunduğunu ve taksonomik sınıflandırmada tamamlayıcı bir kriter olarak değerlendirilebileceğini ortaya koymaktadır.

Aslan Ergenekon Z., Ezer T. (2025). "Spore morphology of *Asplenium* L. (Aspleniaceae) species from Türkiye". Doğa ve Sürdürülebilirlik Derneği, Doğanın Sesi, 8 (15): 30-39



DOĞANIN SESİ



Asplenium trichomanes, Kadıncık Valley, 30.04.2023. © Z. Aslan Ergenekon

GENİŞLETİLMİŞ ÖZET

Araştırmanın amacı ve literatür:

En eski bitki gruplarından birini temsil eden eğreltiler (Polypodiophyta), sporla üreyen tohumuz damarlı bitkilerdir. Dünya genelinde 12.000'den fazla türü ile geniş bir ekolojik uyum ve morfolojik çeşitlilik sergilerler (WFO, 2024; Smith vd., 2006). Bunlar arasında, Aspleniaceae familyasından *Asplenium* L. en büyük ve en çeşitli eğreltiler otu cinslerinden biridir ve Türkiye'de 20 türle, dünya çapında ise yaklaşık 750 türle temsil edilmektedir (POWO, 2025) (Türkiye e-Florası, 2025). *Asplenium* türleri sadece yaprak morfolojisi ve rizom yapısı gibi makroskobik özelliklerde değil, aynı zamanda spor özellikleri gibi mikroskobik özelliklerde de varyasyonlar sergiler. Son yıllarda, spor morfolojisi (özellikle apertür tipi, sporoderm yapısı ve yüzey süslemesi) eğreltiler otu taksonomisinde destekleyici bir karakter olarak önem kazanmıştır (Tryon & Lugardon, 1991).

Bu çalışma, *Asplenium ceterach* L. ve *Asplenium trichomanes* L.'in spor morfolojisini inceleyerek ve karşılaştırarak *Asplenium* cinsinin taksonomik anlayışına katkıda bulunmayı amaçlamaktadır. Çalışmada spor boyutu, şekli, apertür tipi, sporoderm tabakaları (perin ve ekzin) ve yüzey süslemesini incelemek için hem ışık mikroskobu (LM) hem de taramalı elektron mikroskobu (SEM) kullanılmıştır.

Materyal ve yöntem:

Çalışmada 28 Nisan 2023 tarihinde Cehennemdere Vadisi'nden (37°12'15"K-34°27'16"D) toplanan *A. ceterach* ve 30 Nisan 2023 tarihinde Kadıncık Vadisi'nden (37°13'11,46"K-34°38'04"D) toplanan *A. trichomanes* sporları incelenmiştir. Spor preparatları asetoliz yöntemiyle hazırlanmış ve Olympus DP25 kamera sistemine sahip Olympus CX31 ışık mikroskobu kullanılarak yapılan palinolojik incelemeler sonucunda spor morfolojileri tanımlanmıştır. Palinolojik ölçümler (polar eksen, ekvatorial eksen, ekzin ve perin kalınlıkları, apertür uzunluğu) her tür için en az 35 spordan elde edilmiştir. İstatistiksel analizler SPSS Statistics 22 (Sokal & Rohlf, 1969) yazılımı ile gerçekleştirilmiştir. Sporların yüzey süslemeleri ise Niğde Ömer Halisdemir Üniversitesi Merkez Laboratuvarı'nda taramalı elektron mikroskobu (SEM) ile incelenmiştir.

Bulgular:

Mikroskobik gözlemler her iki türün de heteropolar ve oblat şekilli sporlara sahip olduğunu ortaya koymuştur. *A. ceterach* sporları heteromorf olup, çoğunlukla monolettir fakat nadiren trilet sporlara da rastlanmaktadır. Buna karşın, *A. trichomanes* sporları yalnızca monolettir. Elde edilen palinolojik ölçümler ve kantitatif özellikler Tablo 2 ve 3'te sunulmuştur. Yüzey morfolojisi, özellikle de perispor süslemesi, türe özgü desenler göstermiştir: *A. ceterach*, aralarında skabrat alanlar bulunan dalgalı kıvrımlar sergilerken, *A. trichomanes* daha sık dalgalı kıvrımlar ve bu kıvrımların kenarlarında ekinat benzeri çıkıntılar göstermiştir (**Şekil 3-6**).



DOĞANIN SESİ

Tartışma ve sonuç:

Asplenium ceterach'ta hem monolet hem de trilet sporların tespit edilmesi, daha önceki çalışmalarda yaygın olarak bildirilmeyen tür içi değişkenliği vurgulamaktadır (Erdtman, 1957; Lashin, 2012). *Asplenium ceterach*'ın *A. trichomanes*'e kıyasla daha büyük spor boyutu, İrfan ve diğerleri (2022) ve Szkudlarz ve diğerleri (2024) tarafından elde edilen bulgularla uyumludur. Perispor yüzey desenleri, türler arasında net mikromorfolojik ayrımlar sağlayarak taksonomik değerlerini doğrulamaktadır (Tryon & Lugardon, 1991). Genel olarak, spor morfolojisi -özellikle apertür tipleri ve yüzey ornamentasyonu- *Asplenium* taksonomisi için güvenilir karakterler sunmaktadır. Daha geniş tür örnekleme ve bütünleştirici mikroskobik yaklaşımlarını içeren gelecekteki araştırmalar, morfolojik olarak çeşitlilik gösteren bu cinsin sistematığı hakkında daha derin bilgiler sağlayabilir.

INTRODUCTION

Ferns (Polypodiophyta), one of the oldest plant groups in the world, are seedless vascular plants that reproduce by spores and represent an important step in plant evolution. Successfully adapted to terrestrial life, these plants, with more than 12.000 species (WFO, 2024), are distributed across the globe in a wide range of ecological zones, from the humid tropics to arid habitats (Smith et al., 2006). This diversity points to both their ecological adaptability and morphological flexibility.

The Aspleniaceae Newman, an important family in fern taxonomy, is characterized by the genus *Asplenium* L., which contains about 750 species (POWO, 2025). This genus shows great diversity, especially in terms of leaf morphology, rhizome structure, and spore characters. Although it is a cosmopolitan genus, most of its species are distributed in tropical regions. In Türkiye, 20 species are naturally distributed (Türkiye e-Florası, 2025). *Asplenium* species have epiphytic, lithophytic, or soil-dwelling forms and exhibit structural features that can adapt to different ecological conditions.

In recent years, besides macroscopic morphological features, microscopic characters, especially sporangium type, spore morphology and spore wall structure have gained great importance in fern classification. Spore morphology is not only an effective tool for taxonomic distinctions between species, but also provides descriptive data for phylogenetic approaches (Tryon & Lugardon, 1991).

In this study, the spore morphology of two species of the genus *Asplenium* was investigated comparatively: *A. ceterach* L. and *A. trichomanes* L.. The size, shape, aperture types, perispores, and surface ornamentation of the spores were evaluated using both light microscopy (LM) and scanning electron microscopy (SEM), and the differences between the species were revealed in the light of quantitative and qualitative data.



DOĞANIN SESİ

MATERIAL AND METHODS

The specimens of *A. ceterach* and *A. trichomanes* were collected from their natural distribution areas (**Figures 1, 2**). Detailed information on the collection localities is presented in **Table 1**.

Table 1. Locality information and collection dates of the species.

Species	Locality	Date
<i>Asplenium ceterach</i>	Mersin, Tarsus-Çamlıyayla, Cehennemdere Valley, Doğma place, rock bottom, partly shaded, humid, 1600m. 37°12'15"N-34°27'16"E	28.04.2023
<i>Asplenium trichomanes</i>	Mersin, Çamlıyayla, Kadıncık Valley, Ağılıbüke place, roadside, on rock, 740m. 37°13'11,46"N-34°38'04"E	30.04.2023



Figure 1. *Asplenium ceterach*, Cehennemdere Valley, 28.04.2023. ©Z. Aslan Ergenekon



Figure 2. *Asplenium trichomanes*, Kadıncık Valley, 30.04.2023. ©Z. Aslan Ergenekon



DOĞANIN SESİ

Spore slides for palynological analyses were prepared according the standard acetolysis method (Erdtman, 1960). Observations were carried out with an Olympus CX31 light microscope equipped with a 100x apochromatic oil immersion objective and a 10x micrometric periplanar ocular lens. Microphotographs were captured by an Olympus DP25 digital camera system integrated into the microscope.

Considering that the aperture of the spores is located at the proximal pole, measurements of the polar axis (P) and equatorial axis (E) were taken from the equatorial view. In addition, the thickness of the perine and exine layers, as well as aperture lengths, were measured in both polar and equatorial views. Measurements were made on at least 35 spores until a Gaussian distribution was obtained. The data obtained were evaluated in terms of mean value (M), standard deviation (S) and coefficient of variation (Var.); statistical analyses were performed using SPSS Statistics 22 software according to the methodology of Sokal and Rohlf (1969).

Detailed observations of spore surface morphology were conducted using scanning electron microscopy (SEM). SEM imaging procedures were carried out at the Central Research Laboratory of Niğde Ömer Halisdemir University.

RESULTS

Spores of *A. ceterach* and *A. trichomanes* were examined under a light microscope, morphological measurements were made, and the characteristics of the sporoderm layers were described in detail in **Tables 2, 3**. In the description of spore morphology, the terminological criteria proposed by Erdtman (1969), Faegri and Iversen (1975), Blackmore and Barnes (1991), Punt et al. (2007) and Kapp et al. (2000) were used.

Table 2. Morphological measurements of the *Asplenium* spores. P: Polar axis, E: Equatorial axis, M: Mean, S: Standard deviation, Var.: Variation.

Species	Spore shape	P/E	Polar Axis (µm)			Equatorial Axis (µm)		
			M	S	Var.	M	S	Var.
<i>A. ceterach</i>	Oblat	0.74	35.46	±2.40	30.00-53.00	47.60	±2.55	43.00-52.00
<i>A. trichomanes</i>	Oblat	0.69	28.89	±3.93	25.00-33.00	41.91	±2.33	38.00-46.00



DOĞANIN SESİ

Table 3. Morphological observations and measurements of sporoderm layers of the *Asplenium* spores.
M: Mean, S: Standard deviation, Var.: Variation.

Species	Perine (μm)		Exine (μm)		Aperture Length	Aperture Type	Ornamentation
	Max (S)	Var.	M (S)	Var.			
<i>A. ceterach</i>	9.20 (± 1.80)	5.00- 13.00	1.59 (± 0.37)	1.00- 2.00	37.14 (± 3.15)	Monolete, Trilete	Scabrate
<i>A. trichomanes</i>	9.83 (± 1.74)	7.00- 13.00	1.53 (± 0.29)	1.00- 2.00	29.71 (± 2.04)	Monolete	Scabrate

Descriptions of the spores

The *A. ceterach* spores are heteropolar and predominantly monolete, although trilete spores were occasionally observed. In polar view, the spores are oblate in shape (P/E ratio = 0.74; Table 2), whereas in equatorial view, they appear D-shaped (**Figure 3**). The mean polar axis length is $35.46 (\pm 2.40) \mu\text{m}$, and the mean equatorial axis length is $47.60 (\pm 2.55) \mu\text{m}$ (**Table 2**). The aperture length in monolet spores varies between $33.00\text{-}45.00 \mu\text{m}$. The perine reaches a maximum thickness of $9.83 (\pm 1.74) \mu\text{m}$, and the mean exine thickness is $1.53 (\pm 0.29) \mu\text{m}$ (**Table 3**). The perispore exhibits wavy folds, and scabrate ornamentation is present on the surface areas between these folds (**Figure 4**).

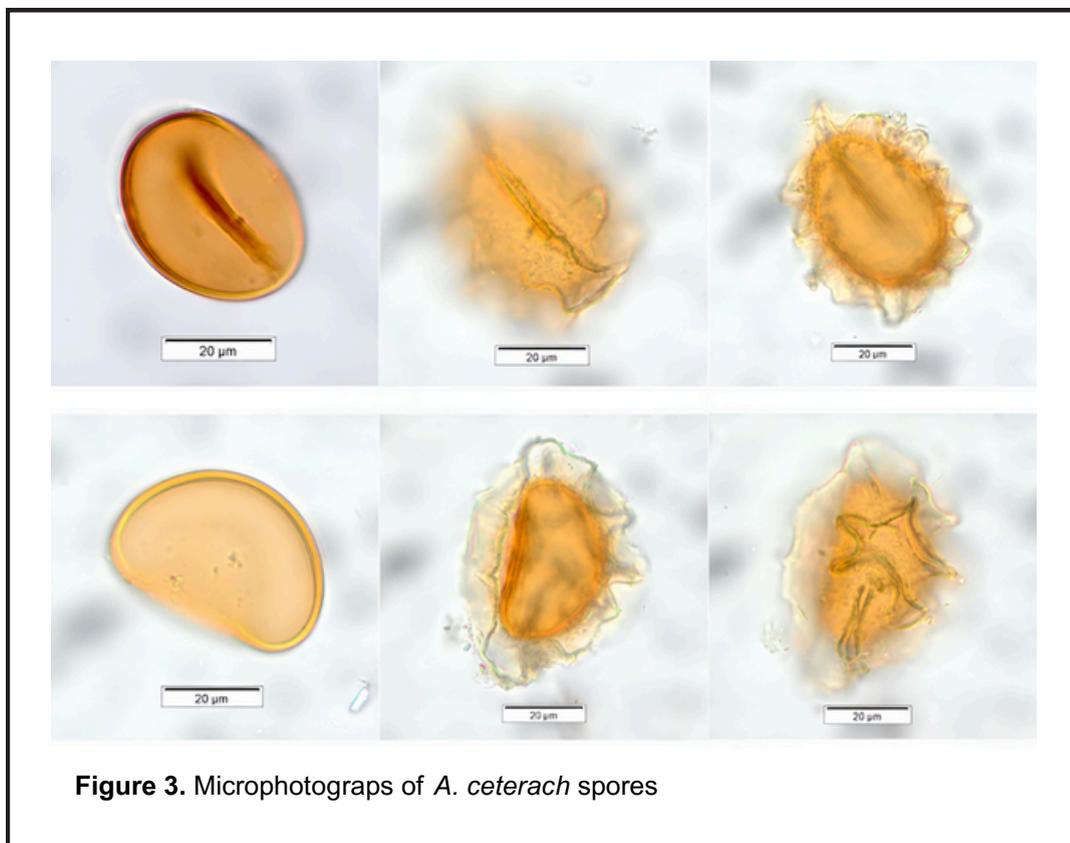


Figure 3. Microphotographs of *A. ceterach* spores



DOĞANIN SESİ

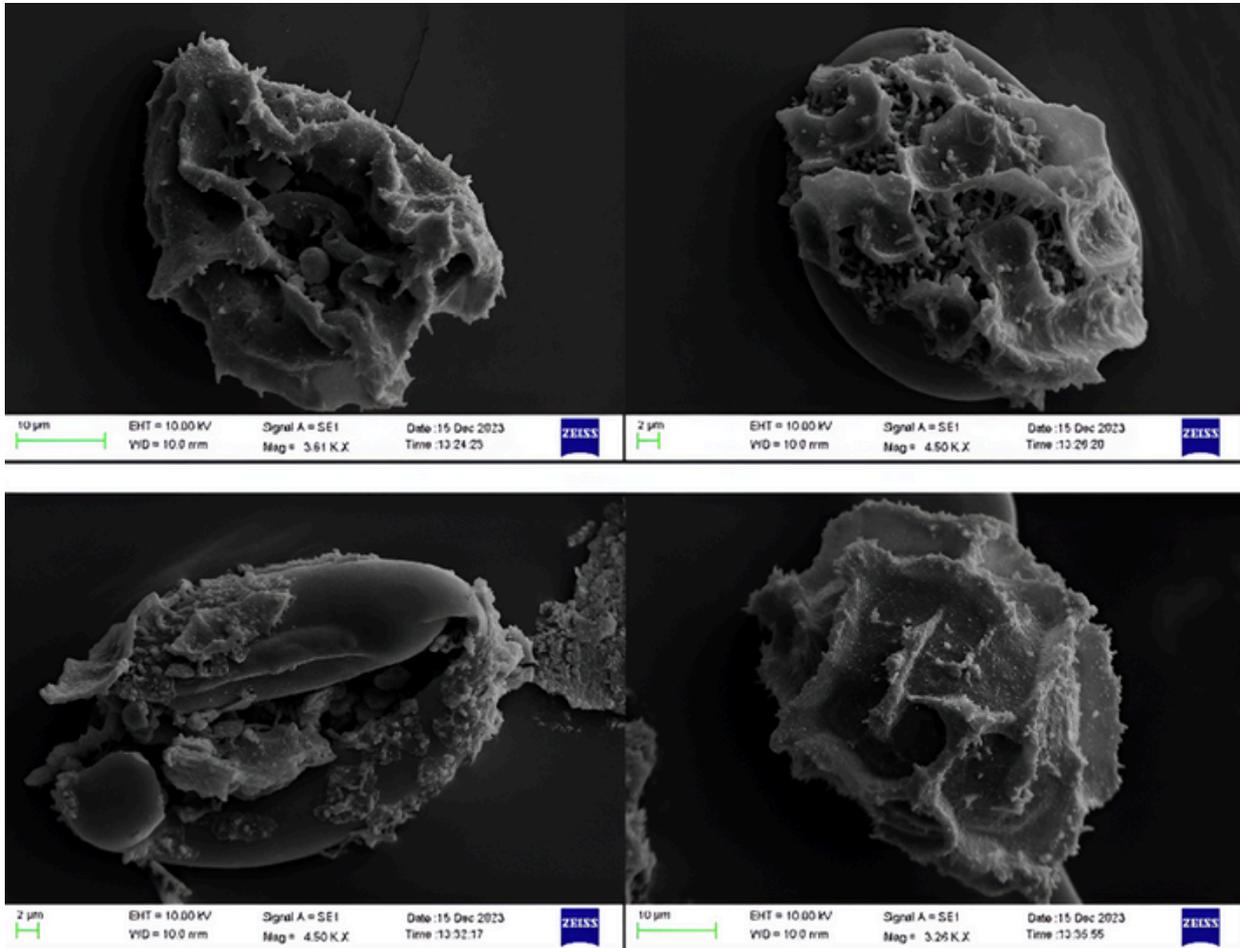
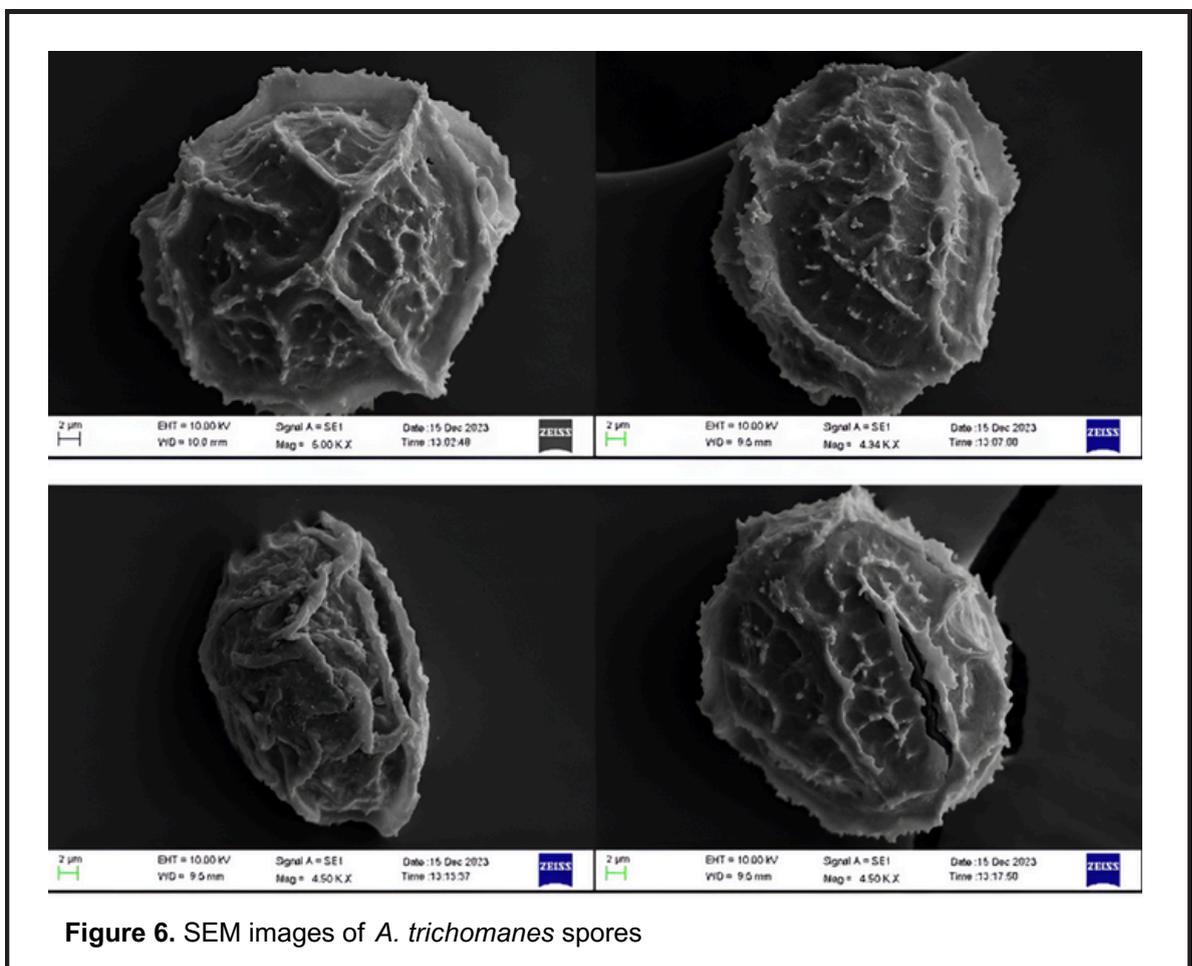
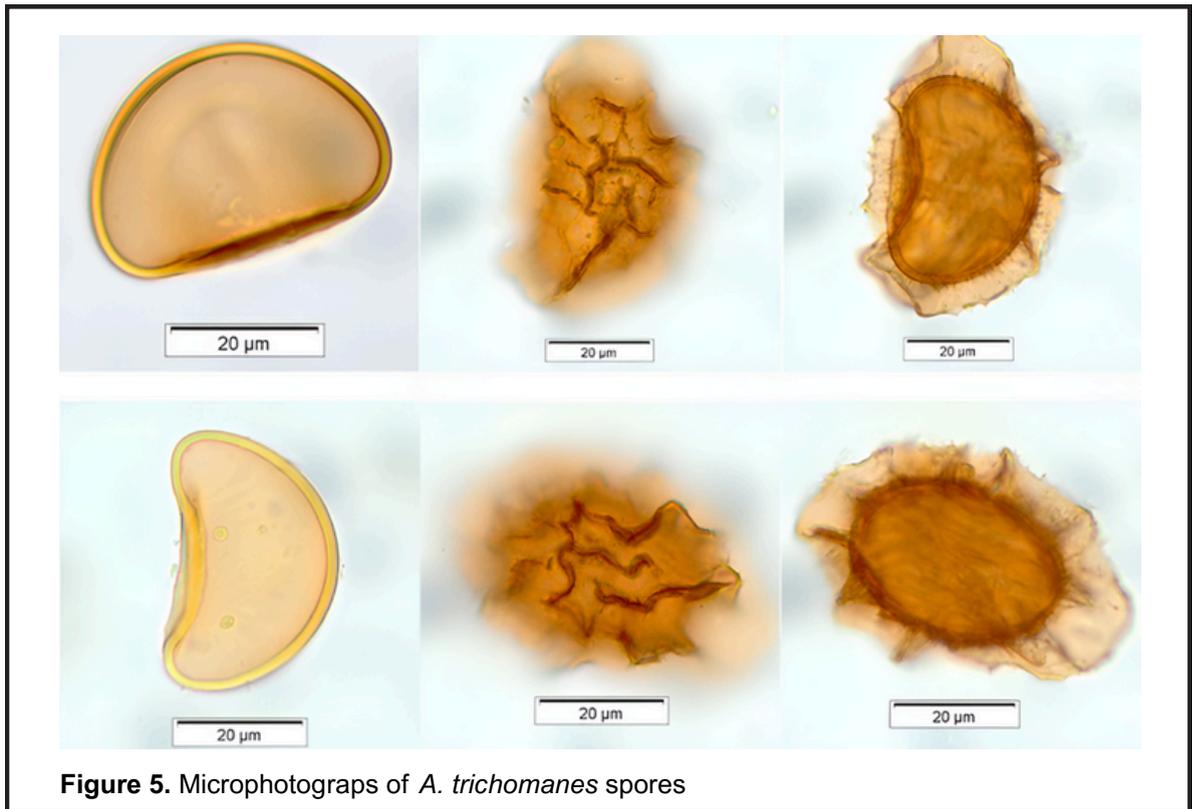


Figure 4. SEM images of *A. ceterach* spores

The *A. trichomanes* spores are heteropolar and monolete. The spores are oblate in shape with a P/E ratio of 0.69 (Table 2). In equatorial view, the spores are reniform to D-shaped (Figure 5). The mean polar axis length is $28.89 (\pm 3.93) \mu\text{m}$, while the mean equatorial axis length is $41.91 (\pm 2.33) \mu\text{m}$ (Table 2). The aperture length ranges between 25.00-35.00 μm . The mean maximum thickness of the perine is $9.83 (\pm 1.74) \mu\text{m}$, while that of the exine is $1.53 (\pm 0.29) \mu\text{m}$ (Table 3).[1] The perispore surface is marked by tightly arranged undulate folds, which are more frequent compared to those observed in *A. ceterach*. There are also echinate-like ornaments on the edges of the folds. Between the folds, finely scabrate ornamentation is present, contributing to the intricate surface texture (Figure 6).



DOĞANIN SESİ





DOĞANIN SESİ

DISCUSSION

In the present study, the spore morphologies of *A. ceterach* and *A. trichomanes* naturally distributed in Türkiye were examined in detail and contributed to taxonomic evaluations. The findings revealed that the spores of both species were heteropolar and oblate-shaped. Although *A. ceterach* spores are mostly monolete, trilete spores were rarely observed, thus indicating that this species has a heteromorphic spore structure. On the other hand, *A. trichomanes* has only monolete spores.

While only trilete or monolete spores are usually reported for *A. ceterach* in the literature (Erdtman, 1957; Lashin, 2012), it is noteworthy that both spore types were found together in the same specimens in the present study. This indicates that the diversity in the spore structure of the species is wider than previously reported and can therefore be considered an important character in taxonomic distinction. Moreover, in terms of spore size, the spores of *A. ceterach* were relatively larger than those of *A. trichomanes*. This finding is consistent with the findings reported by Irfan et al. (2022) and Szkudlarz et al. (2024).

In terms of perineal and exineal layer thicknesses, both species showed similar values, but the presence of more frequent and regular wavy folds on the perispores of *A. trichomanes* spores and echinate-like projections on the edges of the folds reveals a distinct difference in the surface morphology of this species. These observations suggest that spore morphology, especially perispore structure, is an important micromorphological character supporting interspecific discrimination (Tryon & Lugardon, 1991).

In conclusion, spore morphology, especially aperture types and perispore surface features, can be used as distinctive and supportive characters in taxonomic studies of *Asplenium* species. Furthermore, the detailed investigations in this study, conducted through light microscopy and scanning electron microscopy (SEM), once again emphasize the importance of microscopic techniques in the taxonomic and phylogenetic analysis of fern spore structure. In future studies, more comprehensive data on the systematic structure of the genus *Asplenium* can be provided by comparatively analyzing the spore morphology of more species.

ACKNOWLEDGEMENTS

The materials of the study were collected during the field work carried out within the scope of TÜBİTAK project no. 222Z015. We would like to thank the Scientific and Technological Research Council of Türkiye (TÜBİTAK).



DOĞANIN SESİ

REFERENCES

- Blackmore S. & Barnes S. (1991). "Pollen and Spores. Patterns of Diversification". The Systematics Association. Special Vol. No. 44. Clarendon Press, Oxford.
- Erdman, G. (1969). "Handbook of palynology. An introduction of the study of pollen grains and spores". Denmark: Copenhagen.
- Erdtman G. (1960). The Acetolysis Method, A Revised Description, Svensk Botanisk Tidskrift, 39: 561-564.
- Erdtman, G. (1957). "Pollen and spore morphology/plant taxonomy". Gymnospermae, Pteridophyta, Bryophyta (illustrations). (An introduction to palynology. II.).
- Faegri K. & Iversen J. (1975). "Textbook of modern pollenanalysis". Hafner Press, Munksgaard, Copenhagen.
- İrfan, M., Jan, G., Murad, W., & Jan, F. G. (2022). "Taxonomic importance of spore morphology of selected taxa of *Asplenium* (Aspleniaceae) from Pakistan". Microscopy Research and Technique, 85(7):2486-2496.
- Kapp, R., Davis, O., & King, J. (2000). "Pollen and spores, the American association of stratigraphic palynologists foundation". Texas A&M University, USA.
- Lashin, G. M. (2012). "Palynological studies of some species of Aspleniaceae-Pteridophyta". American Journal of Plant Sciences, 3(3):397-402.
- POWO (2025). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. <https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:328226-2#children> (Accessed on: 15 May 2025)
- Punt, W., Hoen, P. P., Blackmore, S., Nilsson, S., & Le Thomas, A. (2007). "Glossary of Pollen and Spore Terminology". Review of Palaeobotany and Palynology, 143:1-81.
- Sokal R. P. & Rohlf J.F. (1969). "The Principles and Practice of Statistics in Biological Research", W.H. Freeman and Company, San Francisco.
- Smith, A. R., Pryer, K. M., Schuettpelz, E., Korall, P., Schneider, H., & Wolf, P. G. (2006). "A classification for extant ferns". Taxon, 55(3):705-731.
- Szkudlarz, P., Celka, Z., Szczeniak, E., Shevera, M. V., & Drapikowska, M. (2024). "Micromorphology of fern spores as a tool in taxonomy of East-Central European species from the family Aspleniaceae (Polypodiopsida)". Acta Societatis Botanicorum Poloniae, 93:187-281.
- Tryon, A. F., & Lugardon, B. (2012). "Spores of the Pteridophyta: surface, wall structure, and diversity based on electron microscope studies". Springer Science & Business Media.
- Türkiye e-Florası. (2025). *Asplenium* L. <https://www.turkiyeflorasi.org.tr/taxon/1b6fca25-b698-425d-aa1e-0ff240fcbb98> (Accessed on: 15 May 2025)
- WFO (2024): World Flora Online. <http://www.worldfloraonline.org>. (Accessed on: 15 May 2025)