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Study some taxonomic aspects of the two species *Limonium meyeri* (Boiss.) Kuntze and *Limonium thouinii* (Viv.) Kuntze, in Iraq

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ABSTRACT: The research aims to separate two species of the genus *Limonium* (*Limonium meyeri* and *Limonium tuinii*) using some Morphological and anatomical characters as taxonomic evidence. The current research included the study of the micro-characteristics of pollen grains and some anatomical characteristics of the stem and leaf using the light and Scanning electron microscope for separation of the species. The attributes of pollen grains (the size of the pollen, the germinating aperture width, and the surface ornamentation). The average length of the polar axis in the polar view reached 45 µm in *L. meyeri* and 35 µm in *L. thouinii*. In addition to the variation in the width of the germination aperture width, which was 17.5 µm in *L. meyeri* and 7.5 µm in *L. thouinii*. as well as the variations in the characteristics of the epidermal cells and the cross-section of the stems, are of good taxonomic importance to separate the two species. The research confirmed that the micro-characteristics of pollen grains and the anatomical characteristics are of complementary taxonomic importance to the morphological traits in separating genera and species.
Keywords: Plumbaginaceae; pollen grains; anatomy of the stem.

Aspectos taxonômicos das espécies vegetais *Limonium meyeri* (Boiss.) Kuntze e *Limonium tuinii* (Viv.) Kuntze, no Iraque

RESUMO: Esta pesquisa objetivou separar duas espécies do gênero *Limonium* (*Limonium meyeri* e *Limonium tuinii)* utilizando alguns caracteres morfológicos e anatômicos como evidência taxonômica. A presente pesquisa incluiu o estudo das micro características dos grãos de pólen e algumas características anatômicas do caule e da folha utilizando o microscópio óptico e eletrônico de varredura para separação das espécies. Os atributos dos grãos de pólen (tamanho do pólen, largura da abertura de germinação e ornamentação da superfície). O comprimento médio do eixo polar na visão polar atingiu 45 µm em *L. meyeri* e 35 µm em *L. tuinii*. Além da variação na largura da abertura de germinação, que foi de 17,5 µm em *L. meyeri* e 7,5 µm em *L. tuinii*. As variações nas características das células epidérmicas e na secção transversal dos caules, foram importantes caracteres taxonômicos para separar as duas espécies. A pesquisa confirmou que as micro características dos grãos de pólen e as características anatômicas são de importância taxonômica complementar às características morfológicas na separação de gêneros e espécies.
Palavras-chave: Plumbaginaceae; grãos de pólen; anatomia do caule.

1. INTRODUCTION

Limonium Miller is one of the largest genera of the Plumbaginaceae family, containing about 350 species, including four in Iraq. Due to its many variations, it is one of the complex genera, as some species have been described as separate species from the genus *Limonium*.

Plumbaginaceae Juss is a family of flowering plants with about 27 genera and 440 species distributed worldwide, mostly in dry areas with saline soils (SINGH et al., 2018). In contrast, Gancedo et al. (2018) indicated that the family includes about 24 genera and 635 species. The family Plumbaginaceae was first described by Antoine Laurent de Jussieu in 1789 (ERDAL, 2015). Darshetkar et al. (2021) and the APG II system group classified the family Plumbaginaceae within the Caryophyllales order. The family Plumbaginaceae is divided into two subfamilies, Plumbaginoideae and Staticoideae, based on phenotypic and chemical characteristics and molecular studies based on plastid DNA sequences (KOUTROUMPA et al., 2019).

The center of diversity for family members of Plumbaginaceae is in the Mediterranean region and the central and western parts of Asia, although it is distributed worldwide. Most members of the family Plumbaginaceae are perennial plants (a few of which are annuals). They grew as trees, shrubs, and herbs. The leaves are petiolate and arranged alternately at the top of the stem, with a rosette arrangement at the base of the stem. Simple, and maybe Pinnate in a few species. The salt glands scattered in the epidermis of leaves and stems exude calcium salts to equalize the salt in the soil. Inflorescences are spikes, raceme, or capitates with bisexual flowers and actinomorphic symmetry. The calyx is gamosepalous and tubular. Corolla is polypetalous, consisting

of five petals joined only at the base, except for some genera in which the corolla is gamopetalous. Flowers have five stamens and a superior ovary with one loculus. Fruits are dry and contain one seed (CAPERTA et al., 2020).

The Limonium genus is known for its diverse chemical compounds, including amino acids, inorganic elements, vitamins, flavonoids, tannins, polysaccharides, alkaloids, and organic acids (BENMEDDOUR et al., 2018). This unique chemical composition adds to the intrigue of our taxonomic study.

Flavonol glycosides, gallates, flavones, flavanones, flavan-3-ols, and gallic acid were isolated from the aerial parts of *L. sinense* (Girard) Kuntze (BAYSAL et al., 2021). Gancedo et al. (2023) reported the presence of hydrolysable and condensed tannins, in addition to the compounds leucoanthocyanins, flavonoids, β-sitosterol, saponins and coumarin in *L. brasiliense* (Antonelli et al., 2015b) showed acute toxicity and safety of the crude extract of *L. brasiliense* rhizomes as it has little or no toxicity in rats and mice, which indicates the possibility of medical use. Yurchyshyn et al. (2017) have demonstrated the efficacy of *Limonium meyeri* (Boiss.) root extract and *Limonium hypericum* Klok as antibacterial and antifungal compared with the aerial parts. Lefahal et al. (2018) have a high content of phenolic and flavonoid components in the aerial parts extract of *L. thouinii,* indicating that it could be used as a sunscreen in pharmaceutical or cosmetic preparations and as a natural source of antioxidants. Gancedo et al. (2023) explained the biological importance of some species of the genus *Limonium*. They praised the importance of the chemical compounds identified for some species of the genus and their effective role as an anticancer in colorectal breast and cervical cancer.

2. MATERIAL AND METHODS

2.1. Morphological study

2.1.1. Preparation of Pollen grains

Pollen grains were prepared in two methods:

i) we prepared the pollen grains using a light microscope. Following the method of AL-Mayah (1983) with some modifications, we boiled the dried flower heads to return them to freshness. They were then preserved in ethyl alcohol (70%) until preparation. The flower heads were opened using a needle, and the pollen grains were spread over a glass slide containing drops of glycerin mixed with safranin dye. The coverslip was gently placed, and the slides were examined under an Optica SN 281166 compound light microscope. Measurements were taken of more than (15) pollen grains for each species, including the axis length (polar and equatorial), using an ocular lens under power (40x) and photographed using a camera mounted on a Zeiss compound microscope under power (40x).

ii) using a scanning electron microscope (SEM), mature, unopened flower heads were taken, and pollen grains were spread directly onto adhesive tape mounted on a glass slide. The slides were transferred to a gold plating machine and then to a stop in a scanning electron microscope.

Photography was done in the electron microscope unit of the College of Science / University of Kufa. The terminology of Erdtman (1971) was adopted.

2.2. Anatomical study

2.2.1. Preparation of Epidermis Cells

The method of AL-Amery (2018) was adopted to prepare leaf and stem epidermis from fresh and dry specimens. Dry samples were placed in boiling water for 1-2 minutes.

The stem and leaf epidermis were prepared using the peeling method and a dissecting blade. The prepared epidermis was placed in a petri dish containing distilled water to remove remaining materials and tissues. The samples were stained with safranin dye prepared at a concentration of 1%.

The prepared epidermis was placed in the middle of a slide containing a drop of glycerin and covered with the slide cover. The stomata and epidermal cells (for twenty samples) were examined and measured under a Zeiss compound microscope using an ocular micrometer at 40X. A photograph was taken using an HD 1080P camera mounted on a Zeiss microscope at 40X.

2.2.2. Preparation of the Stem Cross Sections

This study was conducted on fresh and dry samples. The selected stems were cut into pieces 1-1.5 cm long from the middle of the plant stem. Depending on the stem thickness, the dry pieces were boiled for 3-5 minutes to achieve freshness. After that, the samples were transferred to vials containing an amount of the stabilizer (F.A.A.) Formalin - Acetic acid - Alcohol, which was prepared according to the method of Sass (1958). It was then washed with 70% ethyl alcohol and preserved with the same alcohol and concentration until use.

Then, manual cutting was performed. The stem piece was held vertically between the thumb and forefinger. A sharp dissecting blade was cut into thin pieces and repeated until very thin sections were obtained, with a thickness of (10-15) µm. Safranin stain was used to stain the sections. Then, they were transferred to glass slides containing drops of glycerine and covered gently with the slide cover.

To separate the two species, qualitative characteristics were relied upon. The slides were examined under a compound microscope and photographed using a camera mounted on a Zeiss microscope at 10X.

3. RESULTS

3.1. Pollen grains morphology

Table 1 and Figures 1 and 2 show the information on the pollen grains morphology, where: i) In *L. meyeri*: pollen grains were 3-zonocolporate and medium. In the polar view, the polar axis length (is 37.5 to 55 with a mean of 45 µm). In the equatorial view, the polar axis length (27.5-50) means 45 µm. Equatorial axis length (35-42.5) means 40 µm. The shape of pollen grains in L. Meyer was prolate–spheroidal in the polar view and semi-prolate in the equatorial view, with ornamentation macroreticulater with three germinating pores; ii) In *L. tuinii*: In the polar view, the polar axis length (is 30-37.5 with a mean of 35 µm); in the equatorial view (30-40) mean of 35 µm. and the equatorial axis length (is 22.5-30) means 27.5 µm. The shape of pollen grains in *L. thouinii* was spheroidal in the polar view and semiprolate in the equatorial view, with ornamentation microreticulater with three germinating pores.

Table 1. Variations in the characteristics of pollen grains for the two studied species of *Limonium*.
Tabela 1. Variações nas características dos grãos de pólen das duas espécies de *Limonium*.

Species	Equatorial view (µm)		Polar view (µm)	Germination aperture width
	Polar axis length	Equatorial axis length	Polar axis length	
L. meyeri	(27.5-50.0) 45	(35.0-42.5) 40.0	(37.5-55.0) 45	(15-20) 17.5
L. thouinii	(30.0-40.0) 35	(22.5-30.0) 27.5	(30.0-37.5) 35	(5-10) 7.5

The values inside the brackets represented the minimum and maximum, and those outside the brackets represented the average. 1= *L. meyeri*; 2= *L. thouinii*.

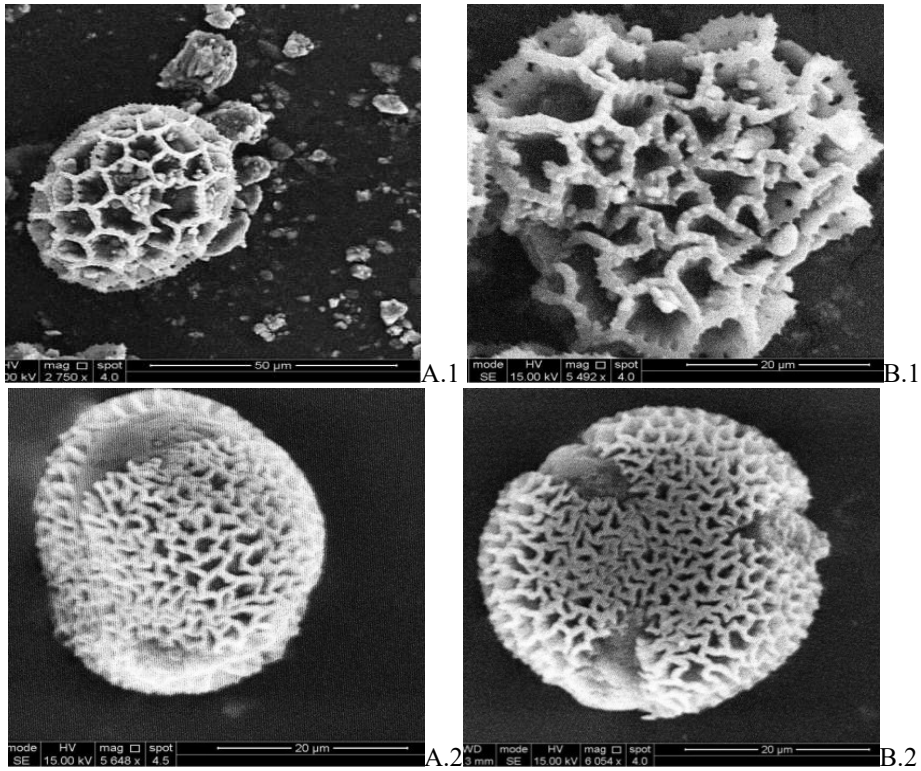

Figure 1. Variations in the shapes and surface decoration of the pollen grains of the two studied species. Under SEM – A: Equatorial view B: Polar view; 1: *Limonium meyeri*, 2: *Limonium thouinii*.
Figura 1. Variações nas formas e superfície dos grãos de pólen das duas espécies estudadas. UnderSEM – A: Vista Equatorial B: Vista Polar; 1: *Limonium meyeri*, 2: *Limonium tuinii*.

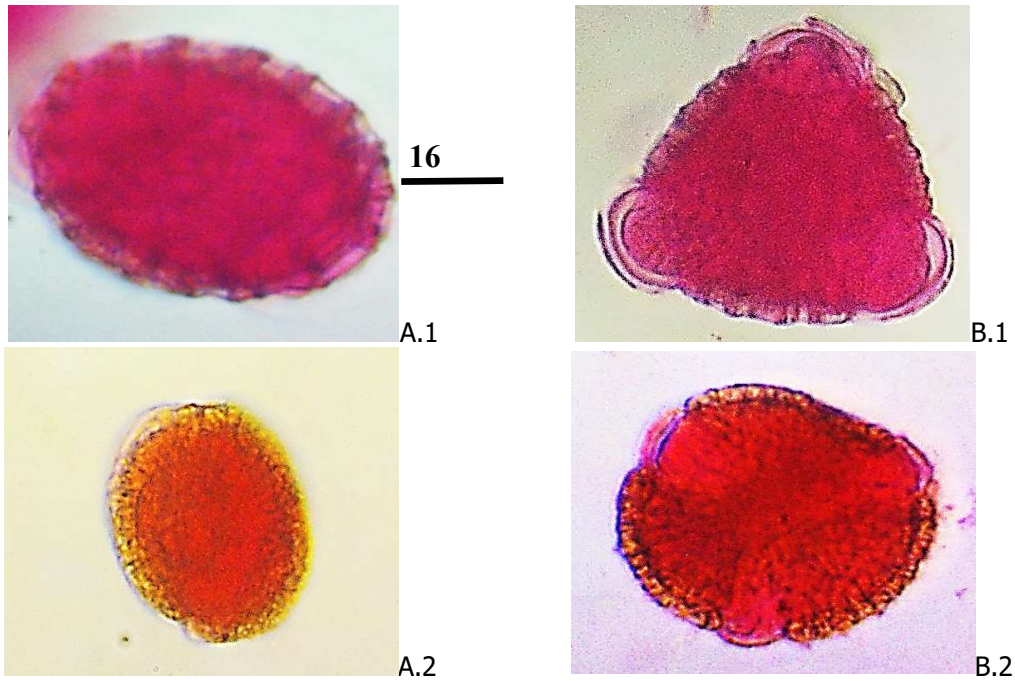

Figure 2. Variations in characteristics of pollen grains for the two studied species. Under light microscope – A: Equatorial view, B: Polar view, 1: *Limonium meyeri*, 2: *Limonium thouinii*.
Figura 2. Variações nas características dos grãos de pólen para as duas espécies estudadas. Ao microscópio óptico – A: Vista equatorial, B: Vista polar, 1: *Limonium meyeri*, 2: *Limonium tuinii*.

3.2. Anatomical characteristics

i) Stem epidermis cells (Table 2): The nature of epidermis cell walls was curved in *L. thouinii* and straight-curved in *L. meyeri* (Figure 3). Average dimensions of epidermal cells reached (65 x 22.5 µm) in *L. meyeri*, while it reached (92.5 x 22.5 µm) in *L. thouinii*.

The dominant stomatal pattern was anisocytic, characterized by three cells of varying sizes surrounding the stomata complex (Figure 3).

ii) Leaf epidermis cells (Table 3): The two species under study had anatomical characteristics characterized by stomata, which spread on the upper and lower surfaces of the leaf. The dominant stomatal pattern was anisocytic. The nature of epidermal cell walls: lower epidermis cells curved in *L. thouinii* and straight-curved in *L. meyeri* (Figure 4). The dimensions of the lower epidermis cells: the upper limit of average cell length is 80 µm in *L. thouinii*, while it reached 60 µm in *L. meyeri*. The upper epidermal cell walls were curved in both species (Figure 5). The cell dimensions: The upper limit of average cell length is 60 µm in L. thouinii, while the lower limit is 55 µm in *L. meyeri*.

iii) Saline glands (semi-circular cells surrounded by several cells arranged in a rosette shape) observed in the leaf epidermis of both species (Figures 4 and 6).

iv) Trichome: Non-glandular (unicellular) hairs were observed on the leaf epidermis of *L. thouinii* and were absent in *L. meyeri* (Figure 7).

v) Stem anatomy: Qualitative characteristics separated the two species (Figure 8). Some taxonomic characteristics were observed to separate the two species, including the winged stem in *L. thouinii*, cortical bundles in species *L. meyeri*, variations in the shape of the stem cross-section, in addition to the number and dimensions of the vascular bundles and their arrangement in the section (Figure 8).

Table 2. The Variations in the characteristics of the epidermal cells and stomata of the stem of the two species of the *Limonium*.
Tabela 2. As variações nas características das células epidérmicas e estômatos do caule das duas espécies de *Limonium*.

Species	Stomata dimensions (µm)		The stomatal pattern (µm)	Average (cells – length x width) (µm)	The nature of epidermis cell walls
	Length	Width			
L. meyeri	(27.5-42.5) 37.5	(25.0-17.5) 22.5	Anisocytic	65.0 x 22.5	Straight-curved
L. thouinii	(42.5-32.5) 35.0	(25.0-20.0) 22.5	Anisocytic	92.5 x 22.5	Curved

The values inside the parentheses represent the minimum and maximum, and outside the parentheses represent the average.

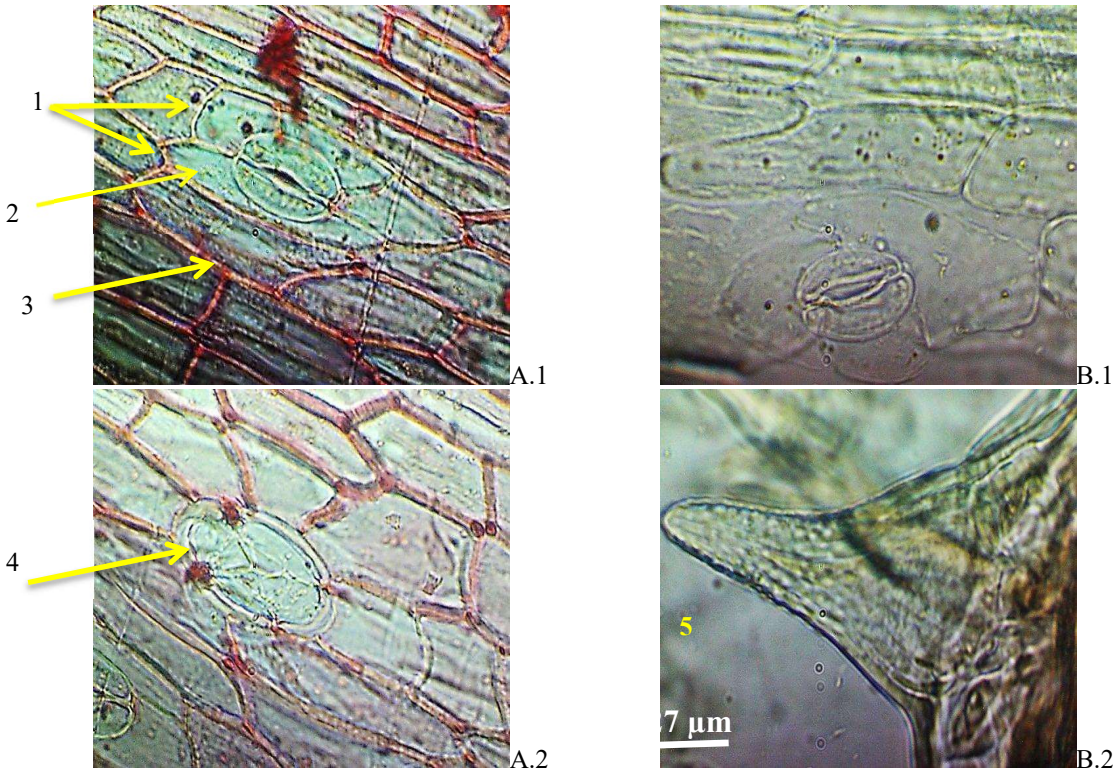

Figure 3. Variations in the characteristics of the epidermal cells and stomata of the stem – A: *Limonium meyeri*; B: *Limonium thouinii*. 1= Subsidiary cells. 2= Guard cell. 3= Ordinary cell. 4= Saline Gland cell. 5=A glandular hair.
Figura 3. Variações nas características das células epidérmicas e estômatos do caule – A: *Limonium meyeri*; B: *Limonium tuinii*. 1= Células subsidiárias. 2= Célula de guarda. 3= Célula comum. 4= Célula da glândula salina. 5=Um cabelo glandular.

Table 3. The Variations in the characteristics of the epidermal cells and stomata of the leaf of the two species of the *Limonium*.
Tabela 3. Variações nas características das células epidérmicas e estômatos da folha das duas espécies de *Limonium*.

Species	Stomata on the upper surface (µm)		Stomata on the lower surface (µm)		The stomatal pattern	Average (cells – length x width) (µm)		The nature of epidermis cell walls (µm)	
	Stoma length	Stoma width	Stoma length	Stoma width		The upper surface	The lower surface	The upper surface	The lower surface
L. meyeri	(27.5-40.0) 32.5	(22.5-25.0) 25	(25.0-37.5) 32.5	(20.0-30.0) 27.5	Anisocytic	55.0 x 30.0	60.0 x 30.0	Curved	Curved
L. thouinii	(25.0-37.5) 32.5	(20.0-25.0) 22.5	(22.5-37.5) 25.0	(20.0-27.5) 22.5	Anisocytic	60.0 x 30.5	80.0 x 37.5	Straight-curved	Straight-curved

The values inside the parentheses represent the minimum and maximum, and outside the parentheses represent the average.

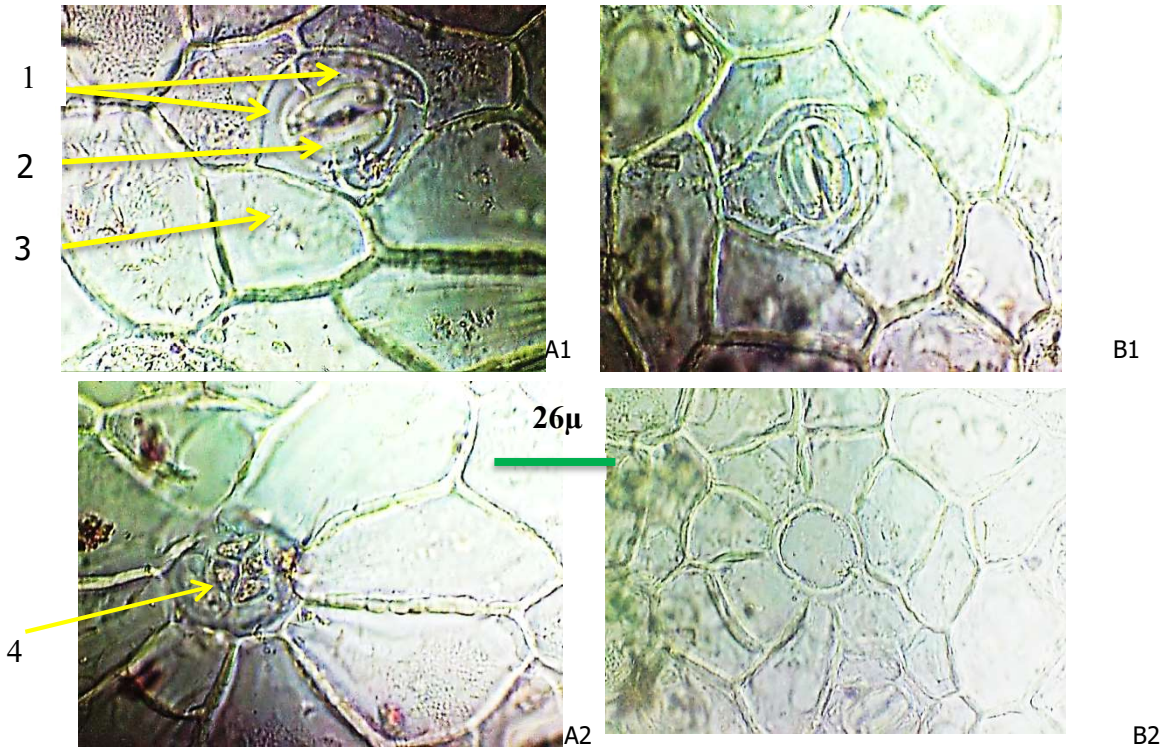

Figure 4. The Variations in the characteristics of the epidermal cells of the leaf. *L. meyeri* – A: lower epidermis; B: upper epidermis. 1= Subsidiary cells. 2= Guard cell. 3= Ordinary cell. 4= Saline Gland cell. B2 = Aglandular hair position.
Figura 4. Variações nas características das células epidérmicas da folha. *L. meyeri* – A: epiderme inferior; B: epiderme superior. 1= Células subsidiárias. 2= Célula de guarda. 3= Célula comum. 4= Célula da glândula salina.

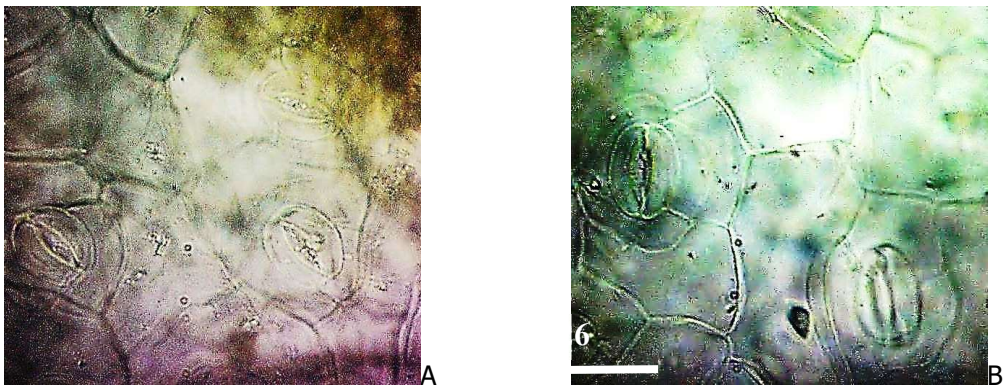

Figure 5. Variations in the characteristics of the epidermal cells of the leaf. *L. thouinii* – A: upper epidermis; B: lower epidermis.
Figura 5. Variações nas características das células epidérmicas da folha. *L. tuinii* – A: epiderme superior; B: epiderme inferior.

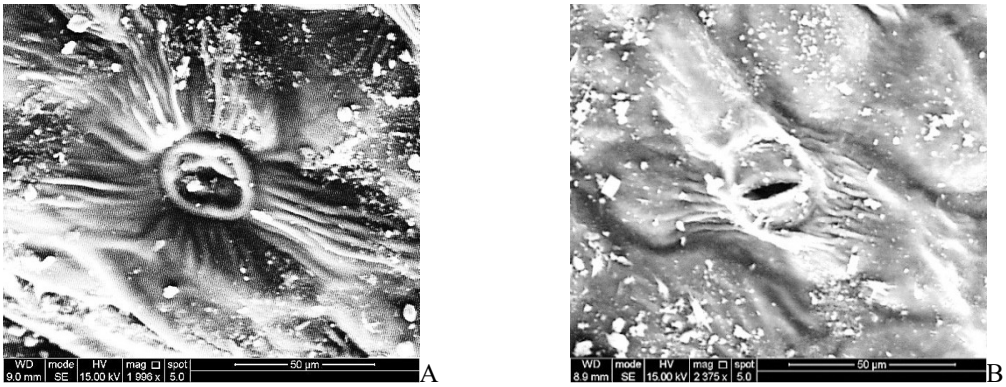

Figure 6. A: Surface view of the saline gland under SEM; B: Surface view of the leaf epidermis.
Figura 6. A: Vista superficial da glândula salina sob MEV; B: Vista superficial da epiderme foliar.

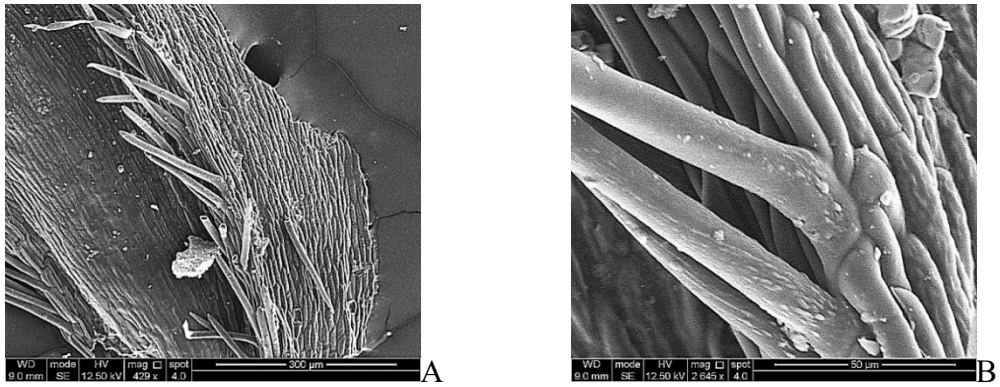

Figure 7. A and B show the shape of the non-glandular hairs scattered on the leaf surface of *Limonium thouinii* under SEM.
Figura 7. A e B mostram o formato dos pêlos não glandulares espalhados na superfície foliar de *Limonium tuinii* sob MEV.

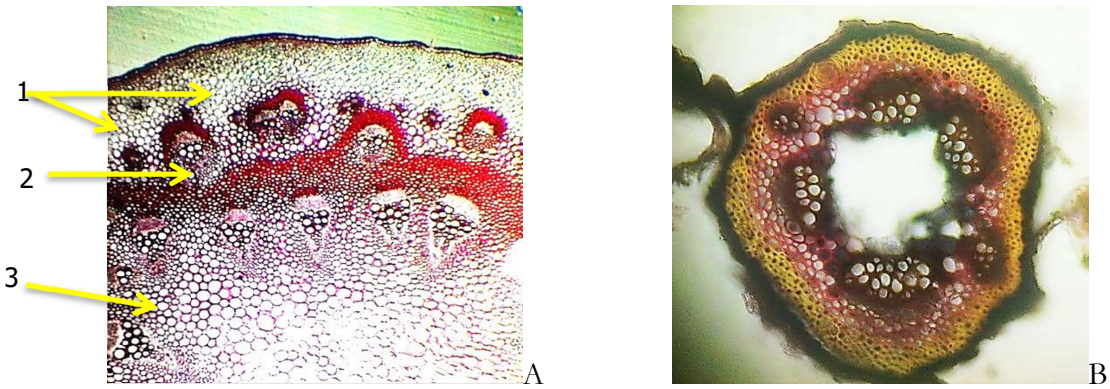

Figure 8. The Variations in the characteristics of the stem cross-section: A: *L. meyeri*; B: *L. thouinii*. 1= Cortical bundles. 2= Sclerenchyma tissue. 3= Pith (Parenchyma tissue).
Figura 8. Variações nas características da seção transversal do caule: A: *L. meyeri*; B: *L. tuinii*. 1= Feixes corticais. 2= Tecido esclerênquima. 3= medula (tecido parenquimático).

4. DISCUSSION

In two species, pollen grains were 3-zonocolporate and medium in size. These results were consistent with the study of Doğan; Baysal (2019). As Walker; Doyle (1975) showed, the Tricolpate is the main and basic type found in eudicots.

Several studies have indicated the taxonomic importance of pollen grain size at the genus level. The current results showed that pollen grains in *L. meyeri* are larger than in *L. tuinii*. This is a good taxonomic feature to distinguish the two species. Either anomaly in meiosis or hybridization causes variation in pollen size (AYTUG et al., 1971). CHANDA et al. (2013) emphasized the taxonomic importance of the size of pollen grains in separating taxonomic units after the characteristics of germination pores and exine ornamentation.

As for the shape of the pollen grain and the number of germination pores, the research results showed that this characteristic has limited taxonomic importance in separating the two species studied. The pollen grain wall (exine) ornamentation was of good taxonomic importance in separating the two species. These results were consistent with the study of (DOĞAN; BAYSAL, 2019). Thus, the characteristics of pollen grains are of good taxonomic importance in distinguishing between the two species.

Anatomical characteristics were also used to separate the two species, as the characteristics of the stem and leaf epidermis were studied, and the characteristics of the cross-section of the stem were clarified.

The results showed a clear difference in the cell walls and the length of the stem cells between the two species in terms of the characteristics of the stem epidermis. Therefore, these are good taxonomic characteristics for separating the two species. These results were consistent with the study of Doğan; Baysal (2019).

As for the anatomical characteristics of the leaf, the dominant stomatal pattern was the anisocytic. In terms of the dimensions of the stomata, the average minimum of the dimensions of the stomata in *L. thouinii* was on the lower surface. At the same time, an overlap was observed in the average dimensions of the stomata on the upper surface in the leaf epidermis of both studied species. Also, during the current study, the spread of non-glandular (unicellular) hairs was observed on the leaf epidermis of L. thouinii, which was absent in *L. meyeri*.

The prevalence of saline glands also characterized the leaf epidermis of both species. It is one of the distinguishing characteristics of *Limonium* leaf epidermis, which was confirmed by González et al. (2021).

Some qualitative characteristics were also used to separate the two species, including the winged stem in *L. thouinii*, cortical bundles in species *L. meyeri*, variations in the shape of the stem cross-section, in addition to the number and dimensions of the vascular bundles and their arrangement in the section. It confirmed the importance of the characteristics of the vascular cylinder in separating species.

5. CONCLUSIONS

The results of the current study clearly show that the micromorphological characteristics of pollen grains and their anatomical characteristics are of good taxonomic importance for separating genera and species.

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Data availability: The data used to verify this study's findings can be obtained by contacting the corresponding author upon request.

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