LEAF MICROMORPHOLOGY OF THE FAMILY PLUMBAGINACEAE IN TURKEY

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LEAF MICROMORPHOLOGY OF THE FAMILY PLUMBAGINACEAE IN TURKEY

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ABSTRACT

LEAF MICROMORPHOLOGY OF THE FAMILY PLUMBAGINACEAE IN TURKEY

Sayar, İpek
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Turkey is located in the region where the species belonging to the *Plumbaginaceae* family mostly spread and it also hosts important endemic species. This study aimed to carry out the leaf micromorphological characteristics of the species belonging to the *Plumbaginaceae* family collected from Turkey. In this study, the surface micromorphology of the dried leaf samples of 9 *Limonium*, 3 *Acantholimon*, 2 *Armeria* and 1 *Plumbago* species were collected by different researchers from all over Turkey and kept at the Plant Systematics Laboratory, Department of Biological Sciences, Middle East Technical University, Ankara were examined by using Scanning Electron Microscope. The data of stoma sizes and density, trichome sizes and density were collected.

Keywords: Plumbaginaceae, Leaf Micromorphology, Stoma, Trichome

ÖZ

TÜRKİYE'DEKİ PLUMBAGINACEAE AİLESİNİN YAPRAK MİKROMORFOLOJİSİ

Sayar, İpek
Yüksek Lisans, Biyoloji
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Türkiye, *Plumbaginaceae* familyasına ait türlerin en çok yayılış gösterdiği bölgede yer almakta ve önemli endemik türlere de ev sahipliği yapmaktadır. Bu çalışmada, Türkiye'den toplanan *Plumbaginaceae* familyasına ait türlerin yaprak mikromorfolojik özelliklerinin belirlenmesi amaçlanmıştır. Bu çalışmada, Türkiye'nin dört bir yanından farklı araştırmacılar tarafından toplanan 9 *Limonium*, 3 *Acantholimon*, 2 *Armeria* ve 1 *Plumbago* türüne ait kurutulmuş yaprak örneklerinin yüzey mikromorfolojisi, Bitki Sistematiği Laboratuvarı, Biyolojik Bilimler Bölümü, Ortadoğu Teknik Ankara Üniversitesi Taramalı Elektron Mikroskobu kullanılarak incelenmiştir. Stoma boyutları ve yoğunluğu, trikom boyutları ve yoğunluğu verileri toplandı.

Anahtar Kelimeler: Plumbaginaceae, Yaprak Mikromorfolojisi, Stoma, Trikom

To my mother

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ABBREVIATIONS

Assadi	Assadi, Mostafa
Boiss	Boissier,François de Sauvages de Lacroix
Bunge	Bunge, Alexander Andrejewitsch
CR	Critically Endangered
EN	Endangered
end.	endemic
et al,	et alii (and others)
Fabr.	Fabricius, Philipp Conrad
IUCN	International Union for Conservation of Nature and Natural Resources
Jaub.	Jaubert, Hippolyte François
Juss	Jussieu, Antoine Laurent de
Kuntze	Kuntze, Carl
L	Linnaeus, Carolus
LC	Least Concerned
Lincz	Linczevski, Igor Alexandrovich
Miller	Miller, Philip
Mobayen	Mobayen, Sadegh
mm	milimeter
Nevski	Nevski, Sergei Arsenjevic
R. Br.	Brown, Robert
Rech. f.	Rechinger, Karl Heinz
sect.	section
Spach.	Spach, Édouard
ssp.	subspecies
var.	variation
Willd	Willdenow, Carl Ludwig
VU	Vulnerable
µm	micrometer

CHAPTER 1

INTRODUCTION

1.1. Brief History and Significance of Taxonomy

It is estimated that there are between 3 and 100 million species on our 4,54-billion-year-old Earth. Almost 8,7 million species are eukaryotic. More than 1,2 million species have been classified since the 1950's, when Carolus Linnaeus began modern taxonomy with Systema Naturae. Still, taxonomic studies continue to identify more species. (Mora, Tittensor, Adl,Simpson & Worm, 2011), (Stevens, 2001)

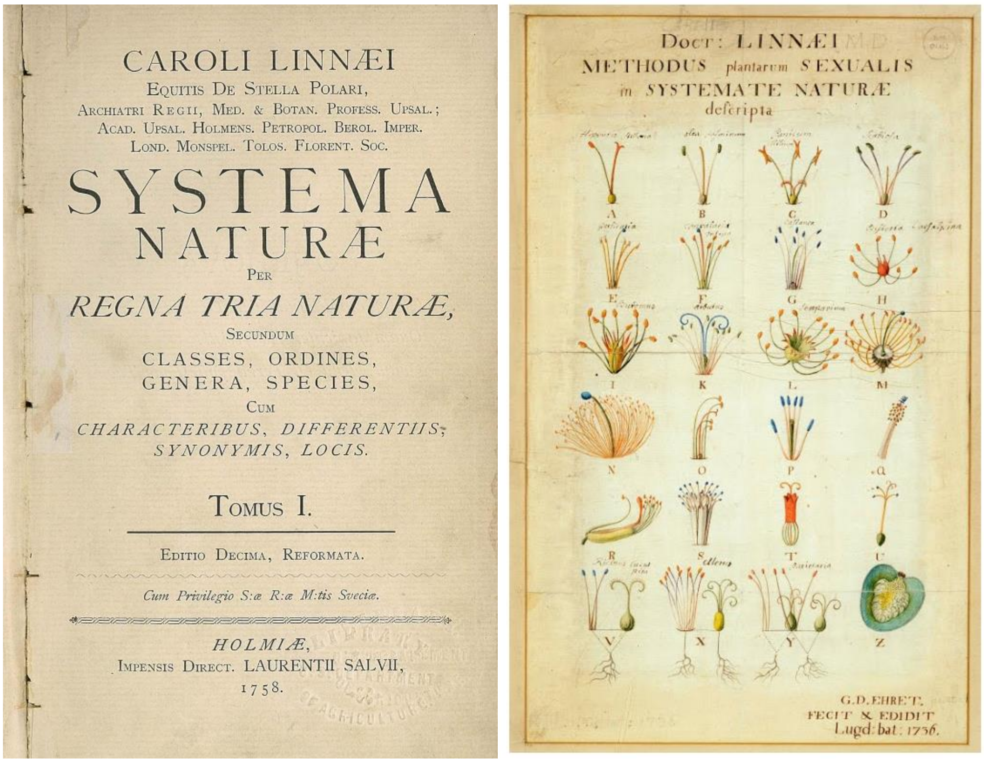

Figure 1: Title page of Systema Naturae (left) and a page of Systema Naturae (right)

Taxonomy includes Description, Identification, Nomenclature, and Classification components. Characters of the species are assigned in the description. In identification, characters of the unknown taxon are compared with the known taxa if it's not new to science. Formal naming of taxa is nomenclature. Taxa are arranged in classification with a certain order. All these processes are crucial for systematics that study all kinds of relationships of species past and present which makes further studies easier and more reliable. (Simpson,2019)

1.2. Micromorphology Studies and Their Importance

Characters that the features of the species or the taxon like petal colour, leaf arrangement, leaf venation type etc. are belong to plant morphology that the study of the external and gross internal structure of plants. (Simpson,2019) Plant morphology studies these structures both with the unaided eye and under the microscope. (Yiğit, 2016) Morphological studies that study the features like root type, stem habit, leaf type, flower symmetry etc. with the naked eye or with a very-low magnification microscope are macromorphological studies, while morphological studies that study pollen grain shape, shape and size of specific cells, trichome types, etc. are micromorphological studies. (Zafer et al, 2019), (Freitas, Reis, L da Costa Bortoluzzi & Santos, 2014)

Micromorphological characters are as important as macromorphological characters in taxonomy and used for the definition and classification of taxa. Scanning electron microscope (SEM) that uses a beam of high-energy electrons to obtain information about the solid surface of the sample by generating signals of them (Omidi et al, 2017) is used to study the plant morphology at micro-levels. (Yiğit, 2016)

Micromorphological studies are efficient in solving taxonomic complications that cannot be solved by the standard morphological studies and in removing ambiguities. (Yigit, 2016) there are known studies that provided exact identification. (Song, Yang & Choi, 2020)

In micromorphological characters, the size, shape, and density of stomata and trichomes are being used for classification. (Ichie et al., 2016)

Stomatal size, shape and density provide information about the habitats and evolutionary process and speciation of the plants because stomatal characters are affected from the density of CO2 , humidity, light and temperature of the environment. (Driesen et al. 1975) (Ichie et al., 2016) (Hong, Lin & He, 2018)

Trichomes as well as stomata are affected from the environmental condition. Some studies have shown that the size, shape and density of trichomes, which have protection against herbivores and environmental conditions such as UV and high temperature, are affected by the characteristics the place where the plants grow. (Ichie et al., 2016)

For this reason, the characters of stomata and trichomes are used in classification while giving information about the region plants live in and their features and intraspecific differentiation.

1.3. The *Plumbaginaceae* Juss. Family

Plumbaginaceae Juss. family which is the only family in the *Plumbaginales* order described by Antoine Laurent de Jussieu (Erdal, 2015) and has 300 to 500 species all over the world. The most species rich and most widespread genus within the *Plumbaginaceae* family is the *Limonium* genus. (Fazlıoğlu, 2011)

Plumbaginaceae family is in the order *Caryophyllales*, and involve 24 genera and 775 species. (Akaydın & Doğan 2006) According to K. Kubitzki, there are 27 genera which are Subfamily Plumbaginoideae; *Plumbago* L., *Plumbagella* Spach, *Dyerophytum* Kuntze, Subfamily Staticoideae; *Aegialitis* R. Br., *Acantholimon* Boiss., *Neogontscharovia* Lincz., *Gladiolimon* Mobayen, *Ghaznianthus* Lincz., *Dictyolimon* Rech. F., *Cephalorrhizum* Popov & Korovin, *Bamiania* Lincz., *Popoviolimon* Lincz., *Chaetolimon* (Bunge) Lincz., *Vassilczenkoa* Lincz., *Limoniopsis* Lincz., *Ikonnikovia* Lincz., *Goniolimon* Boiss., *Bukiniczia* Lincz., *Afrolimon* Lincz., *Bakerolimon* Lincz., *Muellerolimon* Lincz., *Limoniastrum* Fabr., *Limonium* P. Miller, *Eremolimon* Lincz., *Armeria* Willd., *Psylliostachys* (Job. et Spach) Nevski (Kubitzki, 1993) .

6 genera from the *Plumbaginaceae* family that *Plumbago* L., *Limonium* Miller, *Goniolimon* Boiss, *Limoniopsis* Lincz., *Acantholimon* Boiss., *Armeria* Willd. are included in the 7th volume of the Flora of Turkey (Akaydın & Doğan 2006) The genera belonging to the *Plumbaginaceae* family are perennial unlike *Limonium echioides* species. *L. echioides* is the only annual species. (Akaydın & Doğan 2006)

The systematic classification of the *Plumbaginaceae* family:

Kingdom:	**Planteae**
Subkingdom:	**Tracheobionta**
Division:	**Magnoliophyta**
Class:	**Magnoliopsida**
Subclass:	**Caryophyllidae**
Order:	**Caryophyllales**
Family:	**Plumbaginaceae**

1.3.1. The Genus *Limonium* P.Miller

Limonium species are subshrubs or mostly perennial herbs.
Leaves are usually in basal rosettes, but sometimes densely leafy branches might be observed. Inflorescence is a panicle with terminal one-sided spikes that are lax or compact, of 1 up to 12 florets. Calyx is infundibuliform, obconical or tubular, with limb hyaline, 5(rarely 10)-lobed. Corolla is longer than calyx and divided nearly to base, or with a short tube. Styles are free at base. Stigmas are cylindrically filiform. (Kubitzki, 1993),(Davis, 1984)

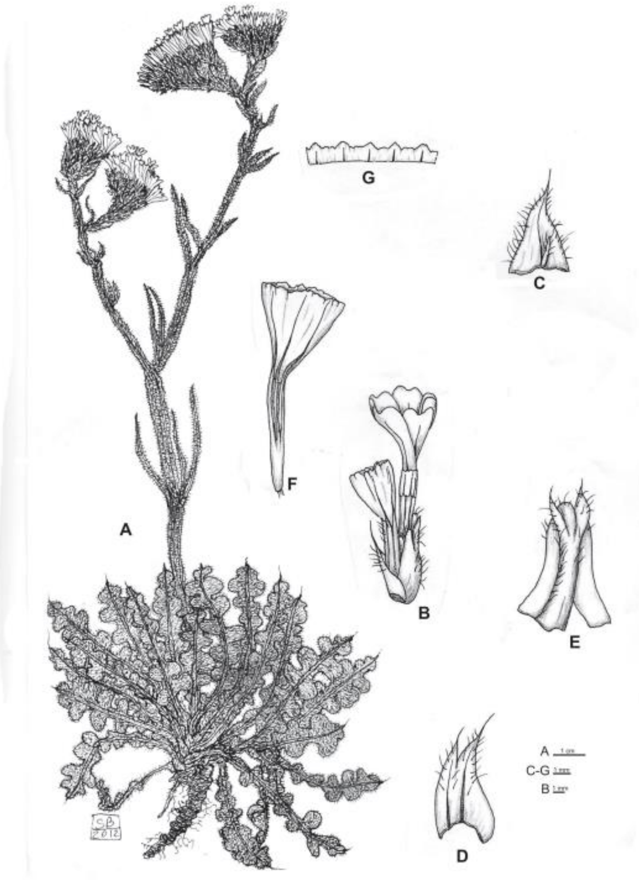

Figure 2: *Limonium sinuatum* (Brullo & Erben, 2016)

In total, there are 19 species of the *Limonium* genus in Turkey.

Limonium Miller is one of the most species-rich and the most widespread genus of the *Plumbaginaceae* family by having 400-500 species. Also, *Limonium* is one of the largest genera in the Mediterranean region (Brullo & Erben, 2016) and have highly rich endemic taxa (70% of the total number of species are endemic) (González-Orenga, Grigore, Boscaiu, & Vicente, 2021)

Limonium genus in European taxa has divided into three subgenera that *Limonium* subgenus *Pteroclados* (~18 species), *Limonium* subgenus *Myriolepsis*, and *Limonium* subgenus *Limonium* by Agnatti. (Lledó, Crespo, Fay & Chase,2005) Subgenus *Myriolepsis* also divided into three sections that *Myriolepsis*, *Siphonantha* and *Polyarthrion*.

Six sections of *Limonium Pteroclados*, *Limonium*, *Schizhymenium*, *Iranolimon*, *Nephrophyllum* and *Sphaerostachys* are the last known sections in Turkey. (Doğan, Akaydın & Erdal, 2020)

Due to the *Limonium* subgenus having have many highly complex and critical taxa, a detailed morphological study is necessary. (Brullo & Erben, 2016)

Limonium species can remove the excess salt by using the glandular cells that found on the plant surfaces. The salt remains after the evaporation of water from the salt glands on the surface of the plant. Then the crystal-formed salts are removed by the different external physical factors. (Brullo & Erben, 2016) With these features, they can survive in areas with high salt concentrations.

Limonium species begin to bloom mostly in summer and continue until winter. (Doğan, Akaydın & Erdal, 2020) (Whipker & Hammer, 1994) (Akaydın & Doğan 2006, p.45)

Table 1: *Limonium* taxa present in Turkey

Subgenus	**Section**	**Species**
L. subgenus *Pteroclados*	*L.* section *Pteroclados*	*L. sinuatum*
L. subgenus *Limonium*	*L.* section *Schizhymenium*	*L. echioides*
	L. section *Iranolimon*	*L. anatolicum*
	L. section *Limonium*	*L. meyeri*
		L. narbonense
		L. effusum
		L. gmelina
		L. marmarisense
		L. vanense
		L. aucheri
		L. gueneri

Table 1 *(continued)*

		L. virgatum
		L. graceum
		L. roridum
		L. sieberi
		L. didimense
	L. section *Nephrophyllum*	*L. bellidifolium*
		L. caspium
		L.smithii
		L. tamaricoides
		L. iconicum
	L. section *Sphaerostachys*	*L. lilacinum* var. *lilacinum*
		L. lilacinum var. *laxiflorum*
		L. pycnanthum
		L. globuliferum var. *globuliferum*
		L. globuliferum var. *subglobosum*
		L. davisii

1.3.1.1. The Distribution of *Limonium*

Limonium species are known as facultative halophytes while a few of them are obligate halophytes. They can grow on soils that are relatively rich in sodium salts concentration and under freshwater. Thus, they prefer to grow in marine coastal habitats that have high salinity in the soil and air. (Brullo & Erben, 2016)

Limonium species are found on different substrates like coarse sands that have good aeration and a low water-holding capacity. (Brullo & Erben, 2016)

14 taxa of *Limonium* are endemic to Turkey. (*L. vanense, L. effusum, L. didimense, L. marmarisense, L. guenerii, L. vanense, L. iconicum, L. tamaricoides, L. lilacinum* var.

lilacinum, var.laxiflorum, L. pycnanthum, L. anatolicum, L. smithii, L. davisii) (Akaydın & Doğan 2006)

1.3.1.2. Economic Importance of *Limonium*

The economic importance of *Plumbaginaceae* includes mostly ornamental cultivars such as *Limonium* (Simpson,2019). The genus is renowned in the international ornamental industry and useful in both fresh and dried. About 15-20 species that include *L. sinuatum* and *L. gmelinii* are used in cultivation. (Morgan& Funnel 2018)

1.3.1.3. Conservation Status of *Limonium*

According to IUCN National Redlist Database, there are many threatened taxa in this genus, and in total 159 species are recorded in red lists (González-Orenga, Grigore, Boscaiu, & Vicente, 2021)

In Turkey, 15 taxa of *Limonium*, *L. vanense, L.ocymifolium, L. graecum var. graecum, var. hyssopifolium, L. caspium, L. tamaricoides, L. lilacinum var. lilacinum, var. laxiflorum, L. pycnanthum, L. anatolicum, L. smithii, L. davisii, L. didimense, L. marmarisense, and L. guenerii* are placed in the category CR, because it is narrowly distributed and not very rich in population. Another 9 taxa *L. sinuatum, L. angustifolium, L. effusum, L. virgatum, L. sieberi, L. bellidifolium, L. iconicum, L. globuliferum* and var. *subglobusum* are placed in the category EN. *Meyeri* and *echioides* taxa are placed in the VU category due to being found only in specific areas and they have a very small population. *Gmelinii* species are placed in the LC category. (Akaydın & Doğan 2006)

1.3.2. The Genus *Acantholimon* Boiss.

Acantholimon species are subshrubs or shrublets. Leaves are subulate, linear, rigid, and pungent. Homomorphic or heteromorphic. Inflorescences is simple or compound distichous spikes with brittle rachises. Spikelets are 1 or 2-5 flowered with 3 or more bracts. Calyx infundibular, rarely tubular. Styles glabrous, rarely verrucose. Stigmas hemispherical or oblong-capitate. (Kubitzki, 1993) (Davis, 1984)

There are 7 sections revised and accepted by Assadi in 2005. These sections are *Acmostegia*, *Acantholimon, Platystegia, Pterostegia, Staticopsis, Tragacanthina* and *Microstegia*. (Erdal, 2015) In Turkey, 3 sections that *Acantholimon*, *Staticopsis* and *Tragacanthina* exist. (Doğan, Duman & Akaydın, 2003) These sections are also divided into many subsections due to difficulties in classification.

Acantholimon Boiss. the genus has 52 species with 10 subspecies and 17 varieties in our country. (Akaydın & Crespo, 2018) According to the latest studies on *Acantholimon* in Turkey, 7 new taxa with 12 new species were revealed. (Akaydın & Crespo, 2018)

Figure 3: *Acantholimon laxiflorum* (Doğan, Duman & Akaydın, 2003)

In the *Plumbaginaceae* family, the *Acantholimon* genus has the most taxa and endemic taxa. 64% of the taxa are endemic. (Akaydın & Doğan 2006)Many *Acantholimon* species are endemic to Turkey. These species are shown in the table. (Akaydın & Crespo, 2018)

The species of this genus prefer to grow in mountainous regions and steppes, on the calcareous and stony soils. (Erdal, 2015) (Kubitzki, 1993) (Ateş ,İğci, Körüklü, Fişne & Aytaç, 2019) However, the species sometimes prefer to live in sea level. (Akaydın & Doğan 2006)

The genus blooms from early spring to late summer (Doğan& Akaydın, 2007)

Table 2: *Acantholimon* taxa in Turkey (Endemic species are marked with "(end.)")

Section	**Species**	**Subspecies**	**Variation**
A. section *Acantholimon*	*A. bracteatum*		
	A. capitatum	ssp. *capitatum* ssp. *sivasicum* (end.)	
	A. hoshapicum (end.)		
	A. bashkaleicum (end.)		
	A. artosense (end.)		
	A. petuniiflorum		
	A. evrenii (end.)		
	A. latifolium		
A. section *Tragacanthina*	*A. tragacanthinum*		
	A. quinquelobum		var. *quinquelobum* var. *curviflorum*
A. section *Staticopsis*	*A. laxiflorum* (end.)		
	A. dianthifolium		
	A. hypochaerum (end.)		
	A. köycegizicum (end.)		
	A. calvertii		var. *calvertii* (end.) var. *glabrum* (end.)
	A. ekimii (end.)		

Table 2 *(continued)*

	A. göksunicum (end.)		
	A. huetii		*var. huetii* (end.) *var. breviscapum* (end.)
	A. turcicum (end.)		
	A. wiedemannii (end.)		
	A. yildizelicum (end.)		
	A. anatolicum (end.)		
	A. strigillosum (end.)		
	A. ulicinum		var. *ulicinum* (end.) var. *purpurascens* var. *creticum*
	A. puberulum	ssp. *puberulum* ssp. *longiscapum* (end.) ssp. *peroninii* (end.)	
	A. karamanicum (end.)		
	A. birandii (end.)		
	A. confertiflorum (end.)		
	A. reflexifolium (end.)		
	A. caesareum (end.)		
	A. caryophyllaceum		
	A. acerosum	ssp. *acerosum*	var. *acerosum* var. *parvifolium* (end.)
		ssp. *brachystachyum* (end.)	
		ssp. *Longibracteolorum* (end.)	
	A. avanosicum (end.)		var. *armenum* var. *balansae*
	A. lepturoides		
	A. kotschyi (end.)		
	A. iconicum (end.)		
	A. halophilum		var. *halophilum* (end.) *var. coloratum* (end.)

Table 2 *(continued)*

A. lycaonicum	ssp. *lycaonicum* ssp. *cappadocicum*	
A. damassanum		
A. hohenackerii		
A. saxifragiforme (end.)		
A. glumaceum		
A. glumaceum		
A. libanoticum		
A. parviflorum (end.)		
A. senganense		
A. spirizianum (end.)		
A. multiflorum (end.)		
A. araxanum		
A. fominii		
A. petraeum		
A. venustum		var. *venustum* var. *assyriacum* (end.)

1.3.2.1 The Distribution of *Acantholimon*

Approximately 200 *Acantholimon* species exist in the world. (Ateş ,İğci, Körüklü, Fişne & Aytaç, 2019) They show a distribution pattern from Southeast Europe to Central Asia. (Doğan, Duman & Akaydın, 2003)

The genus is found in the Eastern Mediterranean and the Irano-Turanian regions in Turkey. (Akaydın & Crespo, 2018)

1.3.2.2. Economic Importance of *Acantholimon*

Acantholimon genus is economically important as ornamental cultivars with attractive long-lasting flowers. (Doğan, Duman & Akaydın, 2003)

1.3.2.3. Conservation Status of *Acantholimon*

26 (39%) taxa are categorized as CR, 15 (22%) of taxa are categorized as VU, 14 (21%) taxa are categorized as EN out of 67 taxa of *Acantholimon* that grown in Turkey according to IUCN (2001) criteria. (Akaydın & Doğan 2006)

1.3.3. The Genus *Armeria* Willd.

Armeria species are perennial herbs with a branched woody root stock. Leaves are simple, linear, arranged in basal rosettes or on densely leafy branches. Inflorescence is a capitulum of aggregate, cymose, 2-4-flowered spikelets at the end of the scape. Calyx infundibuliform, tube 5 up to 10-ribbed. Stigmas is cylindrically, filiform. Fruit with circumscissile or irregular dehiscence. (Kubitzki, 1993)

Only 3 taxa which *A.cariensis* var. *cariensis*, *A. cariensis* var. *rumelica*, *A. trojana* exist in our country. (Akaydın & Doğan 2006)Two native species *A. trojana* and *A. cariensis* in Turkey. *A. cariensis* has two varieties which *A. cariensis* var. *cariensis* and *A. cariensis* var. *rumelica*. (Yeşil, Özhatay&Uruşak, 2014) *A. trojana* species is endemic species in Turkey. (Akaydın & Doğan 2006)

Armeria genus is a taxonomically complex genus due to its high hybridization rate. A high hybridization rate causes a high variation of diagnostic characters and possibility of making boundaries between species. (Erdal, 2015)

They prefer to grow on meadows, mountain steppes, siliceous rocks and stony areas. (Akaydın & Doğan 2006)

The genus blooms from early spring to late summer (Akaydın & Doğan 2006)

Figure 4: *Armeria trojana* (Yeşil, Özhatay&Uruşak, 2014)

Table 3: *Armeria* taxa in Turkey

Species	**Variation**
A. cariensis	var. *cariensis*
	var. *rumelica*
A. trojana	

1.3.3.1 The Distribution of *Armeria*

Armeria genus is found within the North temperate zone and South America, from western Turkey to the Atlantic coasts. (Lefebvre & Vekemans, 1995)

The 3 taxa in our country are grown in the Mediterranean region. (Akaydın & Doğan 2006)*A. cariensis* species are found in Aegean and Marmara regions. *A. trojana* species is found only in Mount Ida (Kaz Dağı) (Akaydın & Doğan 2006)

1.3.3.2. Economic Importance of *Armeria*

While it was formerly grown for medicinal purposes, but now it is grown only for ornamental purposes. (Lawrence, 1947)

1.3.3.3. Conservation Status of *Armeria*

According to IUCN redlist many numbers of species of *Armeria* decreasing. (IUCN Red List of Threatened Species, 2022)

A. cariensis var. *cariensis* and var. *rumelica* species are categorized as EN, while *A.trojana* is categorized as CR in Turkey. (Akaydın & Doğan 2006)

1.3.4. The Genus *Plumbago* L.

Plumbago species are leafy herbs, subshrubs or shrubs. Flowers are in terminal, elongate spikes or racemes. Calyx is tubular. Corolla are funnel-shaped, lobes spreading. Stamens are broadened at the base. Style is filiform and hairy. Capsule circumscissile at the base. (Kubitzki, 1993)

Plumbago genus is the genus that gives its name to the family. (Akaydın & Doğan 2006)

Only one *Plumbago* species grows in Turkey according to 7th volume of Flora of Turkey. (Akaydın & Doğan 2006)

The genus has no endemic species in our country. (Akaydın & Doğan 2006)

Some *Plumbago* species, especially *P. zeylenica* L. have medicinal properties. These species are potential therapeutic phytochemical producers that need further investigation to produce medicines. (Shukla, Saxena, Usmani & Kushwaha, 2021)

It grows in open forests, on arid, pebbly, gypsum, limestone, and volcanic slopes, on the edges of fields. (Akaydın & Doğan 2006)

The genus blooms from early spring to winter. (Akaydın & Doğan 2006)

Figure 5: *Plumbago europaea* (Chaumeton, 1820)

Table 4: *Plumbago* taxa in Turkey

Species
P. europaea

1.3.4.1. The Distribution of *Plumbago*

Tropic regions are the regions where this genus is distributed. (Erdal,2015) Many *Plumbago* species exist especially in the Africa. (Friis, Wilmot-Dear, Edmonson, Wondafrsh & Demissew, 2012)

P. europaea, the only *Plumbago* species grown in our country, grows in the Europe-Siberia phytogeographical region. (Akaydın & Doğan 2006)

1.3.4.2. Economic Importance of *Plumbago*

Plumbago species generally known as ornamental cultivars. (Simpson, 2019)

1.3.4.3. Conservation Status of *Plumbago*

P. europaea in Turkey is categorized as LC according to IUCN (2001) criteria. (Akaydın & Doğan 2006)

1.4. Aim of the Study

There are many valuable *Plumbaginaceae* species in Turkey. Most of them are endemic and have highly complex taxa. These species need a detailed micromorphological study because there is not enough micromorphological information about them for further studies.

The aim of the study was to examine micromorphological characters of the species belong the *Plumbaginaceae* family by using Scanning Electron Microscope (SEM) to reveal essential information that is very crucial for taxonomy.

For this purpose, micromorphology of leaf surface of 9 *Limonium*, 3 *Acantholimon*, 2 *Armeria*, 1 *Plumbago* species gathered from Turkey were examined by using Scanning Electron Microscope (SEM).

CHAPTER 2

MATERIALS AND METHOD

2.1. Plant Material

15 species of *Plumbaginaceae* family given in Appendix X were collected from all over Turkey by different researchers on different dates. All specimens are kept at the Plant Systematics Laboratory, Department of Biological Sciences, Middle East Technical University, Ankara.

2.2. Scanning Electron Microscopy Method

Small pieces of dried adaxial and abaxial leaf surfaces of the chosen species belong to *Limonium*, *Acantholimon*, *Plumbago* and *Armeria* genera fixed on aluminium stabs with double sided carbon tapes. They were coated with 8 nm gold-palladium alloy and imaged with Regulus 8230 cold-field emission (CFE) Scanning Electron Microscope (SEM) with the range of 50X – 2000X magnification to observe micromorphological characters at Eskişehir Osmangazi University ARUM Central Research Laboratory Application Centre. (Kütükalan, 2019)

Stoma and trichome sizes were analysed with Digimizer and ImageJ softwares.

CHAPTER 3

RESULT AND DISCUSSION

3.1. Stomata Micromorphology

Stomata are very important organs due to their functions. Characters of these organs that are responsible for the gas and water exchange, respiration, and photosynthesis, provide information about the phylogeny of the plants. (Hong, Lin & He, 2018) Stomata size and density are important micromorphological characters because they might be unique to some taxa according to studies. (Albert & Sharma, 2013) Thus, they are useful in taxonomy, especially for the delimitation.

Adaxial and abaxial leaf surface of 9 *Limonium*, 3 *Acantholimon*, 2 *Armeria*, 1 *Plumbago* species were examined by Scanning Electron Microscope (SEM). Stoma sizes as stoma length and width, pore length and width, stoma density were studied. Trichome sizes and trichome density of specific species were also studied. All analysis were done for both adaxial and abaxial leaf surfaces.

In all species, stoma width is ranged between 8.03 µm and 25.32 µm, stoma length is ranged between 17.15 µm and 33.47 µm, pore width is ranged between 1.33 µm and 6.56 µm, pore length is ranged between 8.65 µm and 21.69 µm.

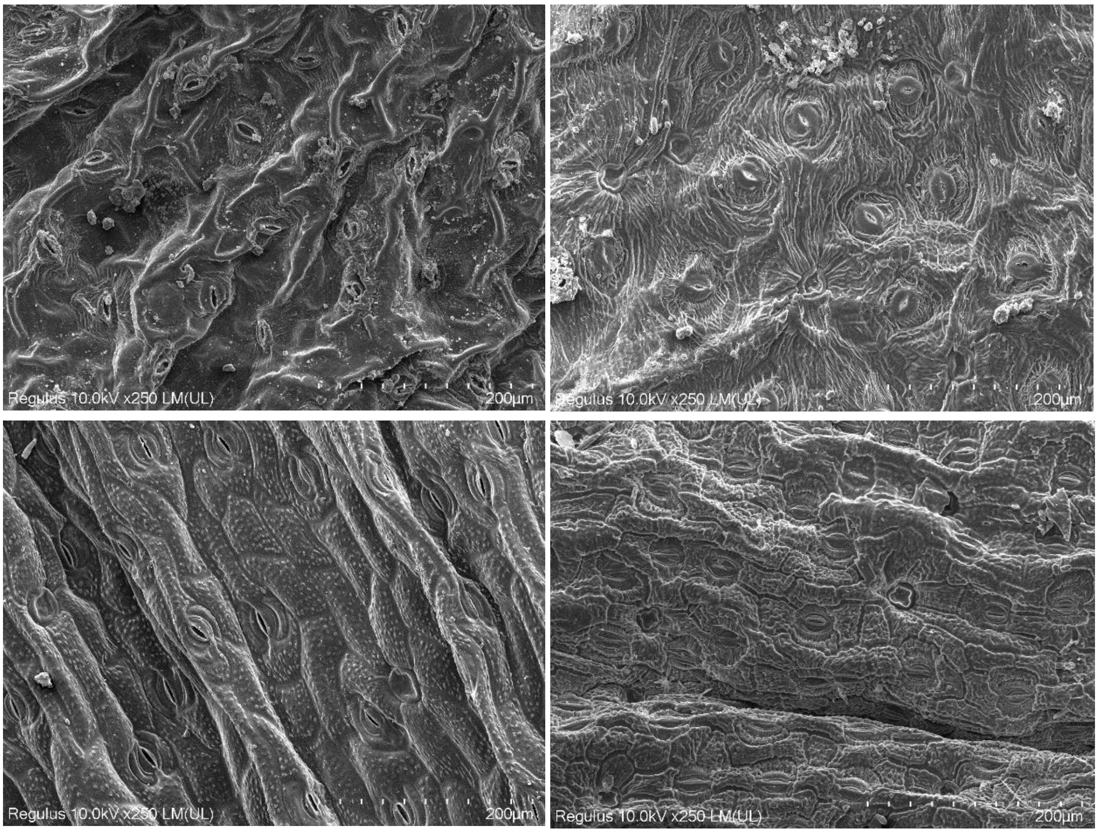

Figure 6: Stoma images of *L.effissium* (scale 200 µm) abaxial leaf surface (Top Left), *L. virgatum* (scale 200 µm) adaxial leaf surface (Top Right), *A. cariensis* (scale 200 µm) adaxial leaf surface (Bottom Left), *A.trojana* (scale 200 µm) adaxial leaf surface (Bottom Right)

The shortest stoma width for adaxial leaf surface is 8.35 µm and belongs to *L. pycnanthum*. The shortest stoma width for abaxial leaf surface is 8.03 µm and belongs to *P. europaea*. The tallest stoma width for adaxial leaf surface is 25.32 µm and belongs to *L. virgatum*. The tallest stoma width for abaxial leaf surface is 24.82 µm and belongs to *L. sieberi*.

The shortest stoma length for adaxial leaf surface is 17.37 µm and belongs to *P. europaea*. The shortest stoma width for abaxial leaf surface is 17.15 µm and belongs to *P. europaea*. The tallest stoma length for adaxial leaf surface is 33.47 µm and belongs to *A. bracteatum*. The tallest stoma length for abaxial leaf surface is 32.85 µm and belongs to *A. sieberi*.

Table 5: Stoma sizes of the *Plumbaginaceae* species examined in this study. (Adaxial Leaf Surfaces are labelled with "(AD"), Abaxial Leaf Surfaces are labelled with "(AB)", The values used in the measurements are in micrometers (µm))

Taxon	Stoma Width	Stoma Length	Pore Width	Pore Length
L. lilacinum (AD)	9.86 ± 1.95 (7.91 – 16,4)	18.48 ± 2.31 (16.17 – 20.79)	4.49 ± 1.99 (2.50 – 6.48)	11.58 ± 3.24 (8.34 – 14.82)
L. lilacinum (AB)	13.81 ± 1.85 (11.96 – 15.66)	26.68 ± 2.35 (24.33 – 29.03)	6.56 ± 1.06 (5.5 – 7.62)	15.11 ± 1.29 (13,82 - 16,40)
L. effisium (AD)	12.54 ± 2.73 (9.81 – 15.27)	25.58 ± 1.77 (23.81 – 27.35)	5.72 ± 1.13 (4.59 – 6.85)	17.76 ± 1.96 (15.8 – 19.72)
L. effisium (AB)	11.40 ± 1.80 (9.60 – 13.20)	26.62 ± 3.44 (23.18 – 30.06)	5.34 ± 1.63 (3.71 – 6.97)	16.20 ± 2.04 (14.16 – 18.24)
L. vanence (AD)	11.65 ± 1.41 (10.24 – 16.06)	21.80 ± 2.44 (19.36 – 24.24)	5.05 ± 1.05 (4.00 – 6.10)	12.92 ± 1.43 (11.49 – 14.35)
L. vanence (AB)	11.60 ± 2.01 (9.59 – 13.61)	21.82 ± 3.70 (18.12 – 25.52)	5.42 ± 1.51 (3.91 – 6.93)	14.26 ± 3.03 (11.23 – 17.29)
L. pycnanthum (AD)	8.35 ± 1.91 (6.44 – 10.26)	18.03 ± 2.60 (15.43 – 20.63)	2.64 ± 1.11 (1.53 – 3.75)	9.91 ± 2.32 (7.59 – 12.23)
L. pycnanthum (AB)	10.45 ± 1.54 (8.91 – 11.99)	19.78 ± 1.80 (17.98 – 21.58)	2.82 ± 1.20 (1.62 – 4.02)	9.66 ± 2.95 (6.71 – 12.61)
L. angustifolium (AD)	13.64 ± 1.86 (11.78 – 15.50)	24.82 ± 2.42 (22.40 – 27.24)	2.44 ± 0.75 (1.69 – 3.19)	13.16 ± 1.92 (11.24 – 15.08)
L. angustifolium (AB)	13.25 ± 2.11 (11.14 – 15.36)	25.34 ± 2.28 (23.06 – 27.62)	2.64 ± 0.76 (1.88 – 3.40)	10.24 ± 1.25 (8.99 – 11.49)

Table 5 *(continued)*

L. sinuatum (AD)	10.09 ± 1.75 (8.34 – 11.84)	22.95 ± 2.29 (20.66 – 25.24)	3.05 ± 0.96 (2.09 – 4.01)	13.37 ± 2.39 (10.98 – 15.76)
L. sinuatum (AB)	N/A	N/A	N/A	N/A
L. virgatum (AD)	25.32 ± 1.34 (23,98 – 26.66)	32.07 ± 2.24 (29.83 – 34.31)	2.40 ± 0.83 (1.57 – 3.23)	9.58 ± 2.18 (7.40 – 11.76)
L. virgatum (AB)	22.87 ± 2.43 (20.44 – 25.30)	29.25 ± 3.06 (26.19 – 32.31)	3.39 ± 1.26 (2.13 – 4.65)	9.83 ± 2.11 (7.72 – 11.94)
L. sieberi (AD)	11.72 ± 1.62 (10.10 – 13.34)	27.63 ± 3.42 (24.21 – 31.05)	3.68 ± 1.68 (2.00 – 5.36)	12.81 ± 4.23 (8.58 – 17.04)
L. sieberi (AB)	24.82 ± 2.98 (21.84 – 27.82)	32.85 ± 2.58 (30.27 – 35.43)	2.81 ± 1.42 (1.39 – 4.23)	8.65 ± 2.44 (6.21 – 11.09)
L. gmelinii (AD)	11.66 ± 1.87 (9.79 – 13.53)	21.43 ± 1.62 (19.81 – 23.05)	1.45 ± 0.24 (1.21 – 1.69)	9.44 ± 2.00 (7.44 – 11.44)
L. gmelinii (AB)	12.05 ± 2.62 (9.43 – 14.67)	23.40 ± 3.41 (19.99 – 26.81)	2.45 ± 0.87 (1.58 – 3.32)	10.93 ± 3.25 (7.68 – 14.18)
A. dianthifolium (AD)	N/A	N/A	N/A	N/A
A. dianthifolium (AB)	N/A	N/A	N/A	N/A
A. wiedemannii (AD)	10.69 ± 2.63 (8.06-13.32)	27.17 ± 2.64 (24.53-29.81)	3.28 ± 0.85 (2.43-4.13)	21.69 ± 2.82 (18.87-23.75)

Table 5*(continued)*

A. wiedemannii (AB)	14.89 ± 1.92 (12.97- 16.81)	25.28 ± 2.66 (22.62-27.94)	1.33 ± 0.35 (0.98-1.68)	15.92 ± 2.06 (13.86-17.98)
A. bracteatum (AD)	13.59 ± 3.25 (10.34- 16.84)	33.47 ± 4.61 (28.86 – 38.08)	2.33 ± 0.65 (1.68- 2.98)	17.96 ± 2.93 (15.03 -20.89)
A. bracteatum (AB)	11.81 ± 1.36 (10.45-13.17)	25.99 ± 3.19 (22.8- 29.18)	1.49 ± 0.65 (0.84- 2,14)	12.78 ± 1.87 (10.91- 14.65)
A. cariensis (AD)	17.41 ± 2.92 (14.49- 20.33)	29.36 ± 1.58 (27.78 -30,94)	1.53 ± 0.39 (1,14- 1,92)	16.82 ± 2.25 (14,57 – 19,07)
A. cariensis (AB)	17.01 ± 1.68 (15.33- 18.69)	27.12 ± 2.00 (25.12-29.12)	1.63 ± 0.46 (1.17- 2,09)	13.49 ± 1.13 (12.36- 14.62)
A. trojana (AD)	12.80 ± 2.59 (10.21- 15.39)	22.29 ± 1.70 (20.59- 23.99)	N/A	N/A
A. trojana (AB)	12.21 ± 1.27 (10.94-13.48)	23.11 ± 2.15 (20.96-25.26)	N/A	N/A
P. europaea (AD)	9.09 ± 1.64 (7.45-10.73)	17.37 ± 2.82 (14.55-20.19)	N/A	N/A
P. europaea (AB)	8.03 ± 1.59 (6.44-9.62)	17.15 ± 3.68 (13.47-20.83)	N/A	N/A

The shortest pore width for adaxial leaf surface is 1.45 μm and belongs to *L. gmelinii*. The shortest pore width for abaxial leaf surface is and belongs 1.33 μm to *A. wiedemannii*. The tallest pore width for adaxial leaf surface is 5.72 μm and belongs to *L. effisium*. The tallest pore width for abaxial leaf surface is 6.56 μm and belongs to *L. lilacinum*.

The shortest pore length for adaxial leaf surface is 9.44 µm and belongs to *L. gmelinii*. The shortest pore length for abaxial leaf surface is 8.65 µm and belongs to *L. sieberi*. The tallest pore length for adaxial 21.69 µm and belongs to *A. wiedemannii*. The tallest pore length for abaxial 16.20 µm and belongs to *L. effisium*.

In all species, stoma density is ranged between 0.048 stomata per μm^2 to 0.193 stomata per μm^2

Table 6: Stomata densities of the *Plumbaginaceae* species examined in this study. (Adaxial Leaf Surfaces are labelled with "(AD"), Abaxial Leaf Surfaces are labelled with "(AB)", Results are given as number of stomata per square of micrometre (μm^2))

Taxon	**Stomata Density**
L. lilacinum (AD)	6/124.609 μm^2 (0.048)
L. lilacinum (AB)	12/124.609 μm^2 (0.096)
L. effisium (AD)	11/124.609 μm^2 (0.088)
L. effisium (AB)	15/124.609 μm^2 (0.120)
L. vanence (AD)	17/124.609 μm^2 (0.136)
L. vanence (AB)	18/124.609 μm^2 (0.144)

Table 6 *(continued)*

L. pycnanthum (AD)	11/124.609 µm^2 (0.088)
L. pycnanthum (AB)	10/124.609 µm^2 (0.080)
L. angustifolium (AD)	10/124.609 µm^2 (0.080)
L. angustifolium (AB)	6/124.609 µm^2 (0.048)
L. sinuatum (AD)	21/124.609 µm^2 (0.168)
L. sinuatum (AB)	N/A
L. virgatum (AD)	10/124.609 µm^2 (0.080)
L. virgatum (AB)	12/124.609 µm^2 (0.096)
L. sieberi (AD)	14/124.609 µm^2 (0.112)
L. sieberi (AB)	19/124.609 µm^2 (0.152)
L. gmelinii (AD)	12/124.609 µm^2 (0.096)

Table 6 *(continued)*

L. gmelinii (AB)	21/124.609 µm^2 (0.168)
A. dianthifolium (AD)	N/A
A. dianthifolium (AB)	N/A
A. wiedemannii (AD)	8/124.609 µm^2 (0.064)
A. wiedemannii (AB)	13/124.609 µm^2 (0.104)
A. bracteatum (AD)	8/124.609 µm^2 (0.064)
A. bracteatum (AB)	9/124.609 µm^2 (0.072)
A. cariensis (AD)	13/124.609 µm^2 (0.104)
A. cariensis (AB)	9/124.609 µm^2 (0.072)
A. trojana (AD)	24/124.609 µm^2 (0.193)
A. trojana (AB)	16/124.609 µm^2 (0.128)

Table 6 *(continued)*

P. europaea (AD)	19/124.609 μm^2 (0.152)
P. europaea (AB)	22/124.609 μm^2 (0.176)

The lowest stoma density for adaxial leaf surface is 0.048 and belongs to *L. lilacinum*. The lowest stoma density for abaxial leaf surface is 0.048 and belongs to *L. angustifolium.*

The highest stoma density for adaxial leaf surface is 0.193 and belongs to *A. trojana.* The highest stoma density for abaxial leaf surface is 0.176 and belongs to *P. europaea.*

Although stomata density is often used for taxonomic purposes, it should not be forgotten that it strongly depends on the environmental conditions in the region where the plant lives. (Agbagwa&Okoli, 2006)

Stomata size and density and pore measurements were used to group genera in other taxonomic studies. However, due to insufficient data, grouping and comparison could not be made in the same way.

3.2. Trichome Micromorphology

Angiosperms are being classified by using amazingly diverse trichomes that one of the plant structures found on the plant surface (Ali & Al-Hemaid 2011) for a long time. These kinds of anatomical information provide great advantage for plant taxonomy. (Nurshahidah et al, 2013)

There are trichomes on the surfaces of the areas of the plant that are in first-order contact with the air. The shapes, locations, and numbers of these trichomes, which have different functions, also differ. Therefore, the morphologies and structures of trichomes are especially important for infrageneric classification within the genus. (Ali & Al-Hemaid 2011)

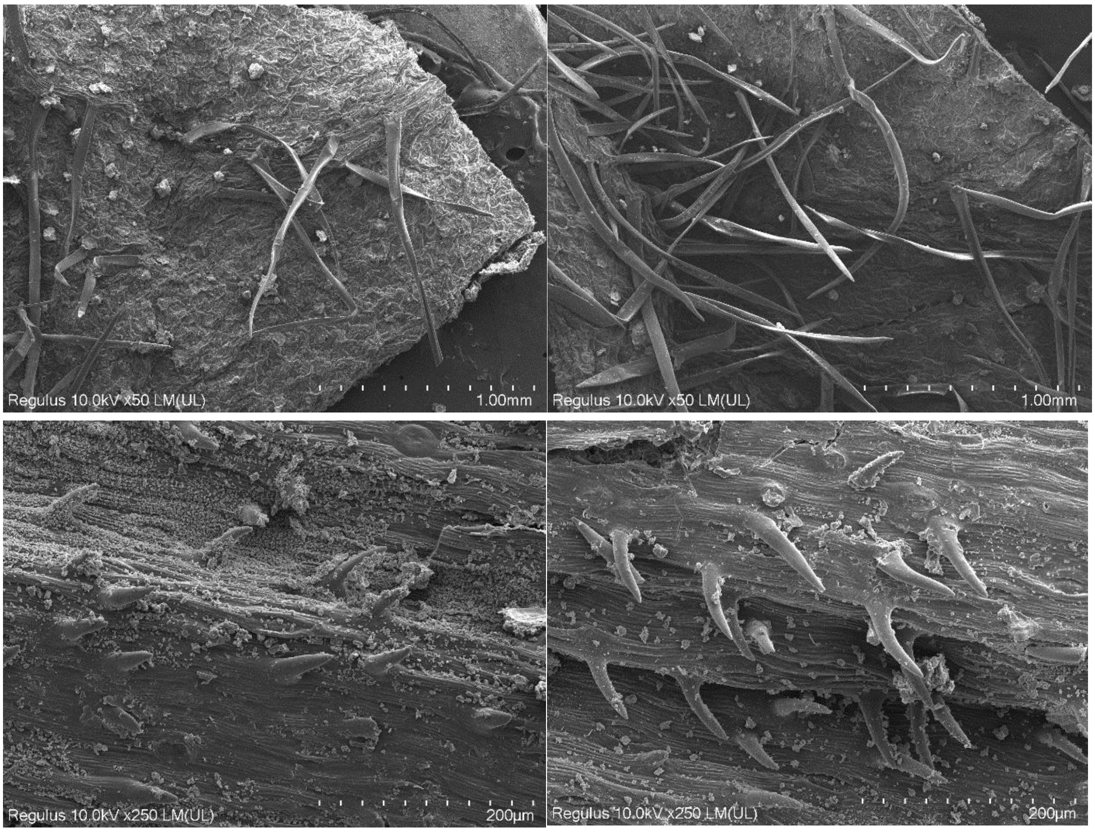

Figure 7 : Trichome images of *L. sinuatum* (scale 1 mm) adaxial leaf surface (Top Left) and abaxial leaf surface (Top Right). Trichome images of *A. dianthfolium* (scale 200 µm) adaxial leaf surface (Bottom Left) and abaxial leaf surface (Bottom Right)

Trichome sizes for adaxial and abaxial leaf surfaces of some species are given in the Table 7. Trichome density for adaxial and abaxial leaf surfaces are given in the Table 8. All Scanning Electron Microscope images of trichomes are illustrated in Figure 7.

Trichomes were observed only on *L. sinuatum, A. dianthifolium, A. widemannii and A. trojana*. In these species, trichome sizes ranged between 49.93 µm to 1.16 mm.

Table 7: Trichome sizes of the Plumbaginaceae species examined in this study. (Adaxial Leaf Surfaces are labelled with "(AD"), Abaxial Leaf Surfaces are labelled with "(AB)", The values used in the measurements are in micrometers (µm))

Taxon	Trichome size (AD)	Trichome size (AB)
A. trojana	N/A	170.78 ± 54.62 µm (116.16-225.4)
A. wiedemannii	53.74 ± 13.83 µm (39.91-67.57)	61.67 ± 12.42 µm (49.25-74,09)
A. dianthifolium	71.44 ± 15.26 µm (56.18 – 86.7)	49.93 ± 10.22 µm (39.71 – 59.22)
L. sinuatum	0.84 ± 0.44 mm (0.4-1.28)	1.16 ± 0.38 mm (0.78-1.51)

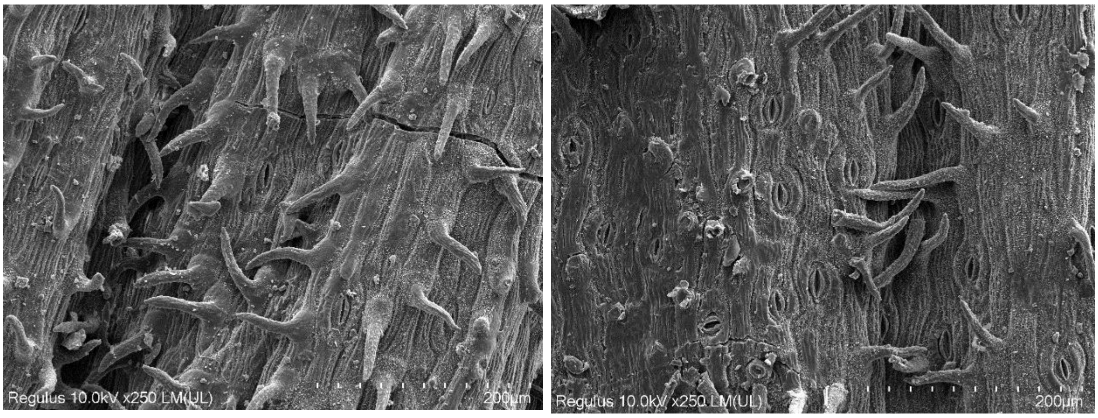

Figure 8: Trichome images of *A. wiedemannii* (scale 200 µm) adaxial leaf surface (scale 200 µm) and abaxial leaf surface (Top Right).

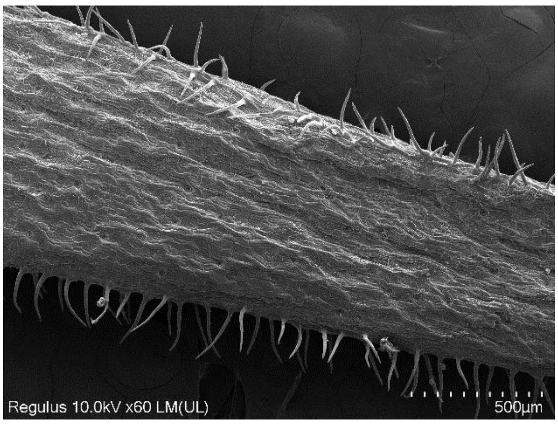

Figure 9: Trichome image of *A. wiedemannii* (scale 500 µm) abaxial leaf surface

In these species, trichome density on adaxial surface ranged between 0.136 (*A. Dianthifolium)* trichomes per µm^2 to 2.873 (*L. Sinuatum)* trichomes per µm^2, on abaxial surface ranged between 0.023 (*A. Trojana)* trichomes per µm^2 to 4.788 (*L. Sinuatum)* trichomes per µm^2.

Table 8: Trichome densities of the *Plumbaginaceae* species examined in this study. (Adaxial Leaf Surfaces are labelled with "(AD"), Abaxial Leaf Surfaces are labelled with "(AB)", Results are given as number of trichome per square of micrometre (µm^2) or trichome per square of millimetre (µm^2))

Taxon	**Trichome Density**
L. sinuatum (AD)	9/3.133 mm^2 (2.873)
L. sinuatum (AB)	15/3.133 mm^2 (4.788)
A. dianthifolium (AD)	17/124.609 µm^2 (0.136)
A. dianthifolium (AB)	14/124609 µm^2 (0.112)
A. widemannii (AD)	30/124,609 µm^2 (0.241)
A. widemannii (AB)	16/124,609 µm^2 (0.128)
A. trojana (AD)	N/A
A. trojana (AB)	34/1471.549 µm^2 (0.023)

Not enough trichomes were observed in this study, where I expect to see more trichomes.

Trichome size and density were used for taxonomic purposes in some studies. However, due to insufficient data, it could not be used in the same way in this study. (Agbagwa & Okoli, 2006) (Saheed & Illoh, 2010)

It is necessary to increase the number of samples in order to be able to obtain enough data. It is important to use plants of the same species and genus, some from the same region. It was not possible to make any comparison with this study, since there is no detailed and recorded study on stomata and trichomes of this species in any other study.

CHAPTER 4

CONCLUSIONS

Since taxonomy is one of the basic parts of systematics, it is a necessity besides being important for the scientific world. It is necessary to be able to understand all living things that have ever existed and will exist in the world, and to continue all detailed studies about them.

There are many methods for identifying and classifying a plant. Some of these give faster and more general results, while others give slower but more precise results. Although precise results are obtained with genetic methods that can be used today, the procedures of these methods take a long time and require a certain budget and equipment. (Dylus et al., 2022) Therefore, morphological studies save time and save budget in the first step. After a certain definition and classification with morphological, especially micromorphological studies, it will be much more productive to direct to genetic studies.

As in other kingdoms, the information obtained as a result of micromorphological studies carried out for classification in the plantae kingdom is very useful for plant classification.

In this family, however, very insufficient micromorphological studies have been carried out. Since there are many *Plumbaginaceae* species in Turkey and most of them are endemic, studies on this family, which is valuable for our country, are of great importance.

For this reason, the species belonging to *Limonium*, *Acantholimon*, *Plumbago* and *Armeria* genera belonging to the *Plumbaginaceae* family collected from Turkey were examined in this study. Stoma sizes (Table 5) and densities (Table 6), trichome sizes (Table 7) and densities (Table 8) were examined in studies with Scanning Electron Microscope (SEM).

Although there was not enough number and variety of species and genera in my study, data for comparison and grouping could not be obtained and other necessary studies could not be found, valuable data were obtained to be used in future taxonomic studies.

Since I believe that such micromorphological studies, which are valuable in terms of classification, should be given importance. This study can be continued by increasing the number of species and samples and can lead to other studies. The obtained results will contribute to future genetic studies and will help to obtain stronger results that can be used in the classification of genera and species belonging to the *Plumbaginaceae* family.

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APPENDICES

A. Specimen Information of Examined Species

Species	Specimen information (Locallity, Date and Collector)
Limonium angustifolium	C2 Antalya, Finike-Kale road, Beymelek surroundings, Seaside thicket (plain) meadows 9-11-2002
Limonium sinuatum	B1 İzmir, Karaburun surroundings. Seaside, cliffs. 8-6-2003
Limonium effisium	B1 İzmir, Urla-Mordoğan eski yolu, Gülbahçe surroundings, Seaside, dunes. 8-6-2003
Limonium lilacinum	B4, Aksaray, University Campus site surroundings, N 38 °20,565' , E 033 ° 58. 505' salt marsh. 950 m. 18-6-2003
Limonium vanence	B9 Van. Van- Muradiye road ~25 Km Saltmarsh, meadows, 1730m. 12-8-2003
Limonium virgatum	C1 Aydın Didim, Akbük, behinde Yar-Sav housing estate. 0m
Limonium sieberi	B1, Balıkesir, Ayvalık, Sarımsaklı surroundings, Salt pits (dunes). 0m 25-6-2003

Limonium gmelinii	C1 Muğla, Datça – Bozburun road, Orhaniye, 0m, 27.07.2004, Doğa & Akaydın
Limonium pycnanthum	B4 Ankara, Şereflikoçhisar – Ankara road, around Tuz lake, 875m, 22.09.2002, Doğan & Akaydın
Acantholimon dianthifolium	C9, Hakkari-Van, old road Bahçeler mevkii surroundings, sedimentary rocks, 2500m. 7-7-2002
Acantholimon bracteatum	B9 Van, Hakkari road Güzeldere pass, calcareous slopes, steppe, 2800m. 6-7-2002
Armeria cariensis	B1 Manisa, Salihli Bozdağ municipality,ski facilities road mountain steppes (meadow) 1550m. 7-6-2003
Armeria trojana	A1- Balıkesir, Edremit. Kazdağı radar road, Kartalçimeni Location, Astragalus steppe. 1750m 6-8-2003
Plumbago europaea	B5 Kayseri, Sultan Sazlığı, Develi-Yahyalı road,Yazıbağları Location,garden. 1071m. 6-8-1994
Acantholimon wiedemannii	B6 Sivas, Yıldızeli, Çamlıbeli da., T.Uverici surroundings sedimentary rocks. Acanth. Union. 1950m 17-8-2001