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FLORAL MICROMORPHOLOGY OF THE GENUS ACANTHOLIMON BOISS. (PLUMBAGINACEAE) AND ITS SYSTEMATICS IMPORTANCE

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

NAMRA KIRAN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

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Submitted by **NAMRA KIRAN** in partial fulfillment of the requirements for the degree of **Master of Science** i**n Biology, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Mesut Muyan
Head of the Department**, Department of Biological Sciences** _____

Assist. Prof. Dr. Musa Doğan
Supervisor, **Department of Biological Sciences, METU** _____

Examining Committee Members:

Prof. Dr.Sertaç Önde
Department of Biological Sciences**,** METU _____

Prof. Dr. Musa Doğan
Supervisor, Department of Biological Sciences, METU _____

Prof. Dr. Ferhat Celep
Biology, Kırıkkale University _____

Date: 07.09.2023

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name Last name: Namra Kiran

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ABSTRACT

FLORAL MICROMORPHOLOGY OF THE GENUS ACANTHOLIMON BOISS (PLUMBAGINACEAE) AND ITS SYSTEMATICS IMPORTANCE

Kiran, Namra
Master of Science, Biology
Supervisor: Prof. Dr. Musa Doğan

September 2023, 69 pages

The aim of this study was to collect taxonomical information for classification by using a scanning electron microscope (SEM) to examine the micromorphological characteristics of the species *Acanthlimon*, a member of the Plumbaginaceae family.For this objective the external floral micromorphology of the calyx, calyx tube, and bracts of 61 *Acantholimon* species from Turkey was investigated using a scan electron microscope (SEM), and we additionally performed cluster analysis. 32 morphological features from 61 species of herbarium specimens were assessed in order to create a data matrix. Software called Multi Variate Statistical Package (MVSP) was used to analyze the data matrix. Phenograms were created using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis. Calyxes, bracts, and hairs were among the micromorphological characteristics of *Acantholimon* that were studied in the study. The data were categorized into three groups based on qualitative and quantitative traits: trichomes, stomata, and surface

sculpuring patterns. Trichomes were discovered on the outer bract, an uncommon *Acantholimon* trait. The remaining species have stomata on the calyx tube and bract, whereas only 2 species of *A. Tragacanthinum* and *A. quinquelobum* have stomata on the calyx. Six surface sculpting patterns were also discovered by the study: the Bract cell surface pattern, the sinuate pattern, the sinuate-reticulate pattern, the reticulate pattern, and the sinuate-sulcate pattern. The investigation in the phenogram revealed clusters. However, few species from other sections are not in close proximity to one another. Hence, to a certain extent, micromorphological characteristics are beneficial for identification.

Keywords: Plumbaginaceae, *Acantholimon.* Floral Micromorphology, Turkey, Systematics importance.

ÖZ

ACANTHOLIMON BOISS (PLUMBAGINACEAE) CİNSİNİN ÇİÇEK MİKROMORFOLOJİSİ VE SİSTEMATİK ÖNEMİ

Kiran, Namra
Yüksek Lisans, Biyoloji
Tez Yöneticisi: Prof. Dr. Musa Doğan

Eylül 2023, 69 sayfa

Bu çalışmanın amacı, Plumbaginaceae familyasının bir üyesi olan *Acanthlimon*'un mikromorfolojik özelliklerini incelemek için taramalı elektron mikroskobu (SEM) kullanarak sınıflandırma için gerekli bilgileri toplamaktır. Bu amaçla Türkiye'den 61 *Acantholimon* türünün kaliks, kaliks tüpü ve braktelerinin dış çiçek mikromorfolojisi taramalı elektron mikroskobu kullanılarak incelendi ve ek olarak küme analizi yapıldı. Bir veri matrisi oluşturmak için 61 herbarium türü örneğinden 32 morfolojik özellik değerlendirildi. Veri matrisinin analizi için Multi Variate Statistical Package (MVSP) adlı yazılım kullanılmıştır. Aritmetik Ortalama (UPGMA) analizi ile Ağırlıksız Çift Grup Yöntemi kullanılarak fenogramlar oluşturuldu. *Acantholimon*'un bu çalışmada incelenen mikromorfolojik özellikleri arasında kalikx, ve kıllar da bulunmaktaydı. Veriler, niteliksel ve niceliksel özelliklere dayalı olarak üç gruba ayrıldı: trikomlar, stomalar ve yüzey yontma desenleri. Nadir bir akantolimon özelliği olan dış brakteler üzerinde trikomlar keşfedildi. Geri kalan türler kaliks tüpü ve brakte üzerinde stomalara sahipken, *A.*

Tragacanthinum ve *A. quinquelobum*'un iki türünün kaliks üzerinde stomaları vardır. Çalışmada altı yüzey şekillendirme modeli de keşfedildi: Brakte hücre yüzey modeli, Sinuate modeli, Sinuate-reticulate modeli, Reticulate modeli ve Sinuate-sulcate modeli. Fenogramdaki inceleme kümeleri ortaya çıkardı. Bununla birlikte, diğer bölümlerden birkaç tür birbirine yakın değildir. Bu nedenle, bir dereceye kadar, mikromorfolojik özellikler tanımlama için faydalıdır.

Anahtar Kelimeler: Plumbaginaceae, *Acantholimon*. Çiçek Mikromorfolojisi, Türkiye, Sistematik önemi

To my beloved family and friends

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CHAPTER 1

INTRODUCTION

Our 4,54-billion-year-old planet is thought to have between 3 and 100 million species. Eukaryotic species number almost 8.7 million. Since the Carolus Linnaeus published Systema Naturae, more than 1.2 million species have been classified. Nonetheless, taxonomic investigations continue to identify new species (Sweeney, 2008).

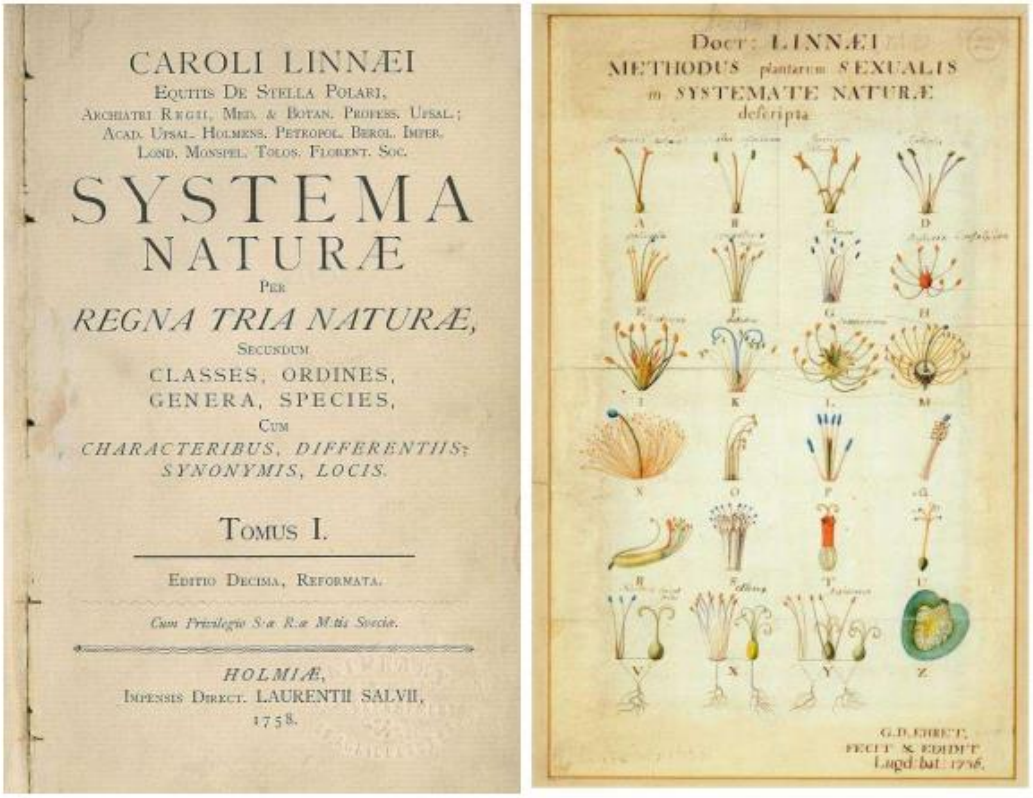


Figure 1-1: Title page of Systema Naturae (left) and a page of Systema Naturae (right).

Since Darwin identified the relationship between the variety of creatures and their shared origin, reconstructing the tree of life has been one of the most contentious aims of the scientific community (Hinchliff et al., 2015).

Description, Identification, Nomenclature, and Classification are all components of taxonomy. The description assigns species characteristics. If the unknown taxon is not novel to science, its characteristics are compared with those of recognized taxa. Nomenclature is the formal naming of taxa. Taxa are grouped in a certain order in categorization. All of these processes are critical for systematics, which analyzes all types of species interactions past and present, making future studies easier and more trustworthy. (Simpson,2019).

1.1 Micromorphological Studies

Plant morphology, the study of the exterior and general interior structure of plants, includes traits that are characteristics of the species or taxon, such as petal color, leaf arrangement, leaf venation type, etc (Simpson, 2019). Both the naked eye and a microscope are used to study these structures in plants. Macromorphological studies are those that examine characteristics such as root type, stem habit, leaf type, flower symmetry, etc. with the naked eye or under a very low magnification microscope, whereas micromorphological studies focus on details such as pollen grain shape, the size and shape of particular cells, trichome types, etc. (Lubna et al., 2019).

In taxonomy, micromorphological characteristics are just as significant as macromorphological characteristics and are used to define and categorize taxa. The study of plant morphology at the microscopic level involves a scanning electron microscope (SEM), which employs a beam of high-energy electrons to generate

signals that may be utilized to gather information about the solid surface of the sample (Yigit, 2017).

Micromorphological investigations are effective at clearing up ambiguities and resolving taxonomic problems that cannot be resolved by conventional morphological research. There are studies that provide precise identification (Yigit, 2017) (Song et al., 2020).

The size, shape, and density of stomata and trichomes are utilized for categorization in micromorphological features (Ichie et al., 2016). Because stomatal characteristics are influenced by the density of CO2, humidity, light, and temperature of the environment, they give information on the habitats, evolutionary process, and speciation of the plants (Hong et al., 2018).

The environment has an impact on both trichomies and stomata. According to several research, the qualities of the environment where the plants are grown have an impact on the size, shape, and density of trichomes, which provide protection against herbivores and harsh climatic factors like UV and high temperatures(Ichie et al., 2016).

Because of this, stomata and trichome characteristics are employed in classification to provide information about the environment in which plants grow as well as about their characteristics and intraspecific difference.

1.2 Systematic Studies on Plumbaginaceae Juss

In his fourth book, "Flora Orientalis", Boissier published the first revisional study of the Plumbaginaceae describing 7 genera and 121 species (Boissier, 1879). Komarov (1967) recognized 11 genera and 131 species in Flora of the USSR, volume 18. Tutin et al. (1972) classified 8 genera and 146 species in the third volume of Flora Europaea. Rechinger and Schiman-Czeika (1974) described 8

genera and 192 species in Flora Iranica. The 15th edition of Flora of China (Wu and Raven, 1996) lists 7 genera and 46 species.

The most important contribution to the study and description of Plumbaginaceae in Turkey was the seventh volume of P.H. Davis' Flora of Turkey and the East Aegean Islands, which had been revised by M.H. Bokhari and J.R. Edmonsdon. In this flora, genera such as *Armeria Willd., Limonium Mill., Acantholimon Boiss*., *Limoniopsis* Lincz., *Goniolimon* Boiss., and *Plumbago* L were recognized. They found 52 species in this family within those genera (Davis et al., 1982, 1988). Furthermore, Bokhari had studied the taxonomy of the South West Asian Plumbaginaceae. He recognized the taxonomic relevance of stigma, pollen types, and anatomical features in addition to revising and describing new species (Bokhari, 1970).

1.3 General Characteristics of Plumbaginaceae Juss

Antoine Laurent de Jussieu described Plumbaginaceae Juss. for the first time in his book Genera Plantarum in 1789. (Jussieu, 1789). The only family in the order was known as the Plumbaginales Lindl. Nevertheless, the Plumbaginaceae plant family has been classified as Caryophyllales Perleb by the Angiosperm Phylogeny Group's (APG II method) of plant taxonomy, which was published in 2003.

The most recent data available has been used to comprehensively classify the Plumbaginaceae family. It belongs to the Kingdom Plantae, which stands for the enormous and varied group of plants. It falls into the Division Magnoliophyta of Plantae, and that's a taxonomic rank used to categorize flowering plants. It is further classified as belonging to the Class Magnoliopsida, a class of flowering plants that includes a wide range of species.

Plumbaginaceae is placed in the Subclass Caryophyllidae, which encompasses numerous plants with distinctive floral structures and other similar characteristics, as we move down the taxonomic the ladder. The family, which belongs to this specific botanical order, is placed in the Order Caryophyllales. Finally, the Plumbaginaceae family was named after the French naturalist Antoine Laurent de Jussieu (Jussieu is sometimes abbreviated as Juss. in botanical nomenclature).

Botanists, researchers, and plant enthusiasts can better comprehend the connections among various plant species and how they evolved with the help of this systematic categorization. It provides a framework for further research and investigation into the distinctive traits and ecological functions of the Plumbaginaceae family in the larger context of the plant world.

The Latin terms plumbum (which means "lead") and agere (which translates as "to resemble") are the source of the common title Leadworts for the Plumbaginaceae group. There are approximately twenty-four genera and 400 distinct species in the Plumbaginaceae Juss. family, sometimes referred to as the leadwort family. (The Plant List, 2017). This is contested since certain studies (APG II, 2003; Simpson, 2010; Renner and Specht, 2011) state as the family possesses 700–800 species (Singh et al., 2018). This family was divided into two subfamilies based on morphological and chemical characteristics: *Plumbaginoideae* and *Staticoideae* (Kubitzki. 1993; Simpson, 2010).

In 1789, Antoine Laurent de Jussieu identified the plumbaginaceae group as the sole member of the order Plumbaginales. However, the APG system of plant categorization placed the Plumbaginaceae family, which has connections to the Polygonaceae family, in the class Caryophyllales in 2003 based on the carnivorous characteristics of some species. Plumbaginaceae are mostly plants, lianas, and shrubs that grow in saline environments (Singh et al., 2018). The Plumbaginaceae are divided into the following genera: *Afrolimon Acantholimon*, *Armeria*,

Aegialitis, Bukiniczia Bakerolimon, Bukiniczia, Chaetolimon, Cephalorrhizum, Ceratostigma, Dictyolimon, Dyerophytum, Eremolimon, Goniolimon Ghaznianthus, Gladiolimon, Ikonnikovia, Limoniastrum, Limonium, Limoniopsis, Muellerolimon, Neogontscharovia, Psylliostachys, \sPlumbagella, Plumbago, Popoviolimon, sPlumbagella, Vasssilczenkoa (Kubitzki, 1993). Myriolimon, which had previously been identified as a part of the genus *Limonium* under the name *Myriolepis* Boiss., has been split and given its own name. (Lledó, Erben, et al., 2005).

Plumbaginaceae taxa are primarily perennial (with a few exclusions that are annual). They can be shrubs, subshrubs, lianas, or herbs. The leaves vary in arrangement like bottom rosettes as well as fascicular rosettes on annual shoots below scapes. The foliage is simple, with a whole margins that is seldom pinnatifid. The petiole of leaves is often tiny or none. On the leaves and stem, chalk glands, which release water and salts to neutralize salt to the soil, are commonly found. Inflorescences that paniculate, subcapitate, increase, or capitate. Actinomorphic, bisexual, and typically placed in bracteate spikelets, flowers are. Calyx appears to be gamosepalous. Possibilities include tubular, subtubular, obconical, or infundibular. It has five segments and between five and 10 ribs.Calyx is hairy and/or glandular in several species. The calyx lip is frequently hyalinized. The corolla is made up of 5 petals that are either entirely free or just attached at the bed. With gamopetalous corollas, the genera *Plumbago* and *Psylliostachys* are exceptions. There are five stamens on a flower. The ovary is superior, contains one loculus, and is linked to the ovule by an extended stalk. It has either five or one style. The stigma possesses five lobes if it is 1 (genera *Plumbago* as well as *Ceratostigma).* The stigma is capitate to conical filiform in appearance. The fruit is stale and membranous, with a single seed. (Davis, et al., 1982, Kubitzki, 1993, Simpson, 2010).

Plumbaginaceae are a species rich, extremely diversified family with a global range, with members primarily found in cooler zooons of the Northern part of the glob. Numerous Plumbaginaceae plants have been utilized as garth decor, and some of them offer therapeutical benefits (Koutroumpa et al., 2018).

1.3.1 The Genus *Plumbago* L

Species of *plumbago* are classified as lianas, shrubs, or plants. Their leaves are simple and symmetrical. The blooms comprise terminal, capitate spikes with three bracts along with one bloom per spikelet. Thick stalked glands including a dentate lip are seen on the tubular calyx. It has gamopetalous corolla. There is a single glandular style including a filiform stigma. (Davis et al, 1982). This genus is found throughout the tropicsregion, particularly in Africa. Eight indigenous *Plumbago* species have been identified in the Flora of Tropical East Africa (Friis et al., 2012). This genus's members are also frequently cultivated as ornamentals

Figure1-2: *Plumbago Eurpea*

The only natural species of *plumbago* in both Turkey and Europe is *plumbago europaea L. Plumbago* capensis Thunb. (also known as *Plumbago auriculata Lam*. due to its auriculate leaves) is another species that is widely distributed along the Turkish coast. While this species is indigenous to South Africa, it is often grown as a garden plant in all climes that are warm. There have also been a few documented escapes from cultivation and naturalization. Blisters, inflammation, itching, toothaches, and skin conditions are all treated with it. Moreover, it has insecticidal, antifungal, anticancer, and antimutagenic properties (Singh et al., 2018).

1.3.2 The Genus *Limonium* Mill

The majority of the members of the *Limonium* group have subshrubs as well herbs, however a few are annuals. Typically, leaves are grouped in base rosettes on it. Some species' leaves cease to remain alive before the blooming season is through. Scapes frequently feature six paniculate or cluster branches, as well as the possibility developing sterile branches. Spikelets in the spicate flower range in number from 1 to 8 to 12 florets. With a hyaline lip, the calyx is infundibuliform, obconical, or tubular. The stigma is filiform and cylindrical (Davis et al, 1982) (Kubitzki, 1993).

Mediterranean area is the hub of *Limonium* variety, however, different parts of the planet with conditions similar to the Mediterranean may also harbor this species. Both costal and salty conditions are neccessary for this family. Sea Lavenders are the popular member of this family.

Figure1-3: *Limonium sinuatum* (Brullo & Erben, 2016)

This is one of the biggest genera of Plumbaginaceae, with over 350 species (Kubitzki, 1993). Additionally, it's a very complicated plant family with ongoing modifications. New species have recently been discovered as well as some are splitting off from this plant group. Despite the fact that Boissier carried out the first in-depth study of *Limonium* in 1848, Miller later distinguished it from the group of plants Statice L.

Since already stated, this genus has a vast and intricate variety of species, making it challenging to achieve stable and accurate sectional differentiation. 32 species in the USSR flora are grouped into the follwing divisions,

- Pteroclados Sauv. & Vindt

- *Platyhymenium Boiss*.
- *Limonium Boiss*.
- *Sarcophyllum* (Boiss.) Lincz.

(At first it was thought that this was Boissier's division of limonium).Separation is mostly accomplished via the use of scape and calyx characteristics. With the exception of Siphonocalyx and the formation of a new section, the Flora of Iran has nearly identical section organization. Rech Nephrophylum. The three groups of limonium according to the flora europea classification are listed below.

- *Pteroclados,*
- *Myriolepis*
- *Limonium* (Erdal & Dogan, 2015).

The subgenus Myriolepis has been promoted to the generic level and given the name Myriolimon, which is the most recent change to the structure of the *Limonium* genus. (Lledó, Crespo, et al., 2005).

17 *Limonium* species have been found in the Turkish Flora and divided into the following five categories:

- *Sphaerostachys*
- *Schizyhymenium*
- *Sarcophyllum*
- *Pteroclados*
- *Limonium*,

The other two species, which Bokhari later included in the list of flora of turkey are listed below

- *Limonium vanense* Kit Tan & Sorger
- *Limonium caspium* (Willd.) Gams (Davis et al, 1982).

Limonium smithii Akaydn (Akaydn, 2007) and *Limonium* gueneri Doğan, Duman & Akaydn (Doan et al, 2008) (Koutroumpa et al., 2018)were recently characterized and published. However there are certain species might represent the noval species are mentioned below

- *Limonium davisii*,
- *Limonium didimense*
- *Limonium marmarisense* (Doğan et al., 2008)

.

1.3.3 The Genus *Acantholimon* Boiss

The species of Acantholimon can be classified as shrubs or subshrubs. This species's leaves a pungent odor and are straight and awl in shape. No matter if it is homomorphic or heteromorphic. The flowers are composed of simple or intricate distichous spikes having fragile rachises. Spikelets have three or more bracts and one to five blooms. Calyx is occasionally tubular but mostly infundibular. morphologies with little verrucae. Stigmas might be oblong-capitated or hemispherical. (Kubitzki, 1993) (Davis, 1984).

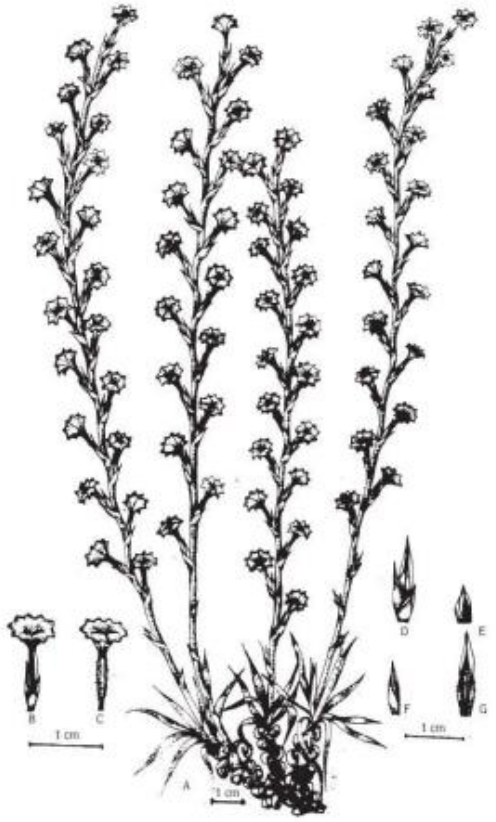


Figure1-4: *Acantholimon laxiflorum* (Doğan, Duman & Akaydın, 2003)

There are around 165 species in the genus (Kubitzki, 1993). Its distribution area extends from southern Greece through Turkey, Iran, Afghanistan, and west Tibet. This genus has a high rate of endemism. The majority of the species are local endemics with a limited geographical area. As was already mentioned, *Limonium* has the highest number of species, but *Acantholimon* follows closely in second place with the highest number of species. Similar to *Limonium*, *Acantholimon* is a fairly complex genus with a wide range of traits across its species. As a result, making grouping among the genera, which makes seperation challenging.

In 1846, Boissier classified the Statice L. subgenus Armeriastrum Jaub. & Spach, formerly known as acantholimon, as a distinct genus, and he named it as follows.

- *Armeriopsis*
- *Staticopsis*
- *Glumaria* (Moharrek et al., 2014)

Here are the seven sections that ASSADI divided them into after reviewing them in 2005. There are seven sections:

- *Acmostegia*
- *Acantholimon*
- *Platystegia*
- *Pterostegia*
- *Staticopsis*
- *Tragacanthina*
- *Microstegia*

There are three sections in Turkey: *Acantholimon, Staticopsis,* and *Tragacanthina*. Due to categorization issues, these parts are also divided into several subsections (Doğan et al., 2003).

Boiss, *Acantholimon*. In turkey, there are 52 species, 10 subspecies, and 17 variations. According to the most recent *Acantholimon* research in Turkey, 7 new taxa and 12 new species have been discovered (Crespo, 2018).

Acantholimon has the most taxonomic and endemic taxa in the Plumbaginaceae family (Akaydın & Doğan 2006). Sixty-four percent of the taxa are endemic. Numerous *Acantholimon* species are only found in Turkey (Crespo, 2018).

These genus' members tend to grow in hilly areas and steppes, on calcareous and rocky soils (Erdal & Dogan, 2015) (ATEŞ et al., 2019). Nonetheless, the species occasionally prefers to survive at sea level (Crespo, 2018).

1.3.3.1 The Distribution of *Acanthlimon*

There are around 200 *Acantholimon* species in the world. They are distributed from Southeast Europe to Central Asia (ATEŞ et al., 2019). The genus is found in Turkey's Eastern Mediterranean and Irano-Turanian areas (Doğan et al., 2003).

1.3.3.2 Significance of *Acantholimon*

The decorative varieties of the Acantholimon, which have stunning, lengthy flowers, are important commercially.

1.3.3.3 Phytogeographic Distribution

Because to the vast variety in temperature and geography, Turkey is split into three phytogeographic areas. The Euro-Siberian, Mediterranean, and Irano-Turanian regions are among them n (Davis at all, 1965).

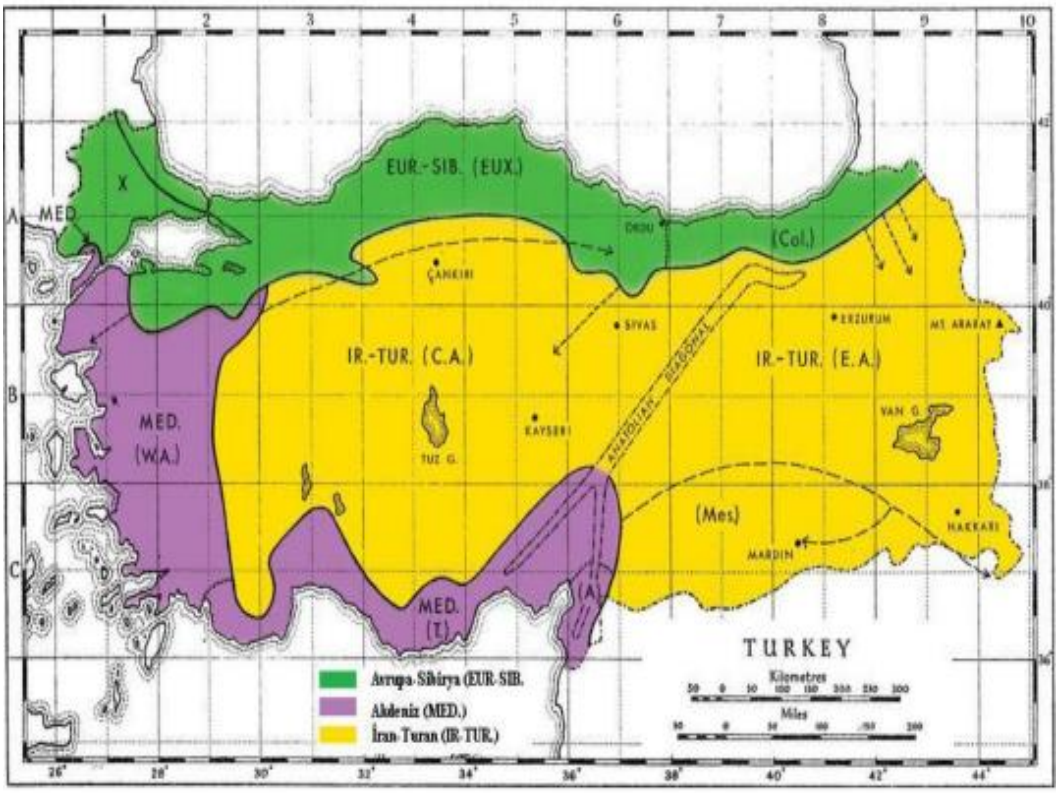


Figure1-5: Phytogeographic regions of Turkey (Erdal & Dogan, 2015)

The greater part of North Anatolia and the European portion of Turkey's little Black Sea coast fall within the Euro-Siberian area. This area experiences a very humid and wet environment. Deciduous woods makes up the vegetation, which gradually transforms into conifer forest as the height climbs. These environmental conditions are not favorable for this family's continued existence. Resources are insufficient in this type of environment for the growth of plumbaginaceae. Only the limonium and plumbago species from this family may thrive in this type of habitat. Only 3.61% of Turkey's total Plumbaginaceae are represented by this.

The Gallipoli peninsula, as well as West and South Anatolia, comprise Turkey's Mediterranean area. As is well known, the Mediterranean climate has scorching,

dry summers and highly wet, forest-free winters. The 31 macchie that are evergreen, aromatic shurbs were replaced by conifers at higher elevations (Davis at all, 1965). This region is home to 5 *Acantholimon* species, 3 *Armeria* species, 1 *Goniolimon* species, and 9 *Limonium* species, It makes up 21.69% of the Turkish Plumbaginaceae.

Nevertheless, when it comes to plant species, the Irano-Turaninan area is the most diverse. This area includes the Esteren Anatolian and central regions. Mountains and highland plateaus divide it. This area has a highly continental climate that eventually changes to semi-desert in certain areas. his region experiences a lot of snowfall every year, which is the main precipitation source. Throughout the spring, there is a lot of rain. Moreover, open plateau environments are frequently quite windy, which increases dryness and has an impact on tree development. Temperature fluctuations are significant, both on a yearly and daily basis. In areas where moisture levels permit, this sort of harsh climate supports flora dominated by herbaceous plants, especially grasses which create prairie and shrubs that might migrate into rare, open conifer forests. Late spring and early summer are prime months for vegetation development., Turkey's Irano-Turanian region is separated into two vegetation zones, which are as follow

1. a huge outside region of uncommon park-like woodland and deciduous scrub.
2. the inner parts of true steppe devoid of trees.

The greatest part of the grassland region is the Central Anatolian steppe, which finally moves eastward into the East Anatolian highlands steppes or mountain protect vegetation (Davis at all, 1965).

This is the finest region in Turkey for Plumbaginaceae growing. As a result, two *Limoniopsis* species, ten *Limonium* species, 49 *Acantholimon* species, and one

Psylliostachys species are found in this region. It is equivalent to 61 species, representing 74.7 percent.

1.3.3.4 The Enviromental Prefrences of the Plumbaginaceae

The first kind of environment includes coastal environments such as rocky terrain, sandy shoreline, and particularly salty marches, which include nearly all *Armeria* species and half of the *limonum* species.

32 species of *Limonium*, or the other half, favor salty inland areas, as do numerous *Acanthalimon* species. *Acantholimon* is primarily found on dry, rocky, or rocky slopes in hills and mountains. Several species can be found in mountainous steppes. But if we're talking about *plumbago eurpea*, you may locate this species in uncultivated, limestone slopes, volcanic craters, and untamed plains. Nonetheless, goniolimon incanum can be found in serpentine, uncultivated plains, and chalky slopes.

1.3.3.5 Endemism of Plumbaginaceae

44 of the 83 Plumbaginaceae species that are now identified as existing are native to Turkey (53.11%). There are also eight unique types and six endemic subspecies.

- *Acantholimon* contains the greatest number of endemic species, with 33 species (Turkey is home to about 61% of the species in the *Acantholimon* genus.)
- *Limonum* has 9 endemic species (42.86%)
- *Limoniopsis* has one endemic species, and the name of the species is *L. Davisii.*

- The only endemic species in the genus *Armeria* is *A. trojana*.

The majority of endemic species are found in the Irano-Turanian area, mainly in the mountain ranges within South Eastern Anatolia. The Irano-Turanian region, particularly the mountain ranges of South East Anatolia, has the greatest number of endemics. This area has 38 endemic species, accounting for 86.36% of all Turkish Plumbaginaceae endemics. *Acantholimon, Limonium*, and *Limoniopsis* are among the 30 species. In the Mediterranean area, the number of endemics is smaller. This region is home to 3 endemic *Acantholimon,* 1 endemic *Armeria,* and 9 endemic *Limonium* species, making up 13.64% of all native in this family. No indigenous Plumbaginaceae from the Euro-Siberian region have been reported.

1.3.3.6 Conversation Status of the Plumbaginaceae in Turkey

According to IUNC, there are several species that are endangered. Below are the classification (Erdal & Dogan, 2015).

Table 1-1 According to IUNC endangered species

CR (Critically Edangered)	EN (Endangered)	NT (Near Thretend)	LC (Least Concern)	VU (Vulnerable)
37 (44.58%)	13 (15.66%)	5 (6.02%)	12(14.46%)	17 (20.48%)

1.4 Objective

Turkey is home to several important Plumbaginaceae species. The majority of them are endemic and have extremely complicated taxonomy. These species require a

careful micromorphological examination since there isn't much micromorphological information available for further research.

The objective of this study was to use a scanning electron microscope (SEM) to analyze the micromorphological traits of the species *Acanthlimon*, which is a member of the Plumbaginaceae family, in order to gather crucial data for categorization and infrageneric grouping in genus *Acantholimon*.

Using a scanning electron microscope (SEM), the external floral micromorphology of the calyx, calyx tube, and bracts of 61 *Acantholimon* species from Turkey was examined.

CHAPTER 2

MATERIALS AND METHOD

2.1 Plant Material

Alarge part of the samples studied in this thesis were collected during the researches on ‘ Turkiye’nin *Acantholimon* Boiss. (plumbaginaceae) Turlerinn Revizyon” (199T011) AND “ Turkiye’deki plumbaginaceae juss”, which were supported by the scientific and research ccouncil of turkey (TUBITAK) (Dogan and Akaydin 2003). After these original reaserchers were completed, the collected materials and specimens were stored in the Plant Systematics Laboratoryin the Middle East Technical University (Ankara, Turkey) as herbarium materials. Certains pecimens of *Acantholimon* were also collected from the Royal Botanic Garden , Edinburgh, which were later also safely stored in the collection.

After their collection, these samples were then studied and classified after an in-depth comparison with similar floras in, firstly, Turkey and East Aegean Islands,secondly, with flora Iranica,then Flora *Europaea*, and fnally, with the flora found in the USSR(Davis at al,1992,1998) (Rechinger and Schiman-Czeika, 1947) (Tutin et al,1972) (Komarov,1967).

In this study, *Acantholimon* samples that were taken between 2000 and 2003 as part of the TBAG-1781 (199 T011) numbered TUBITAK project and kept in the systematic botany laboratory at the Department of Biological sciences, Middle East Technical university (Ankara, Turkey) were utilized.

2.1.1 Method of the Study

Flowers from each taxon were dehydrated in an acetone series, critical-point-dried with carbon dioxide, mounted on the stubs using double-sided adhesive tape, and sputter coated with gold - palladium in order to analyze the surface characteristics of the calyx tube and bracts (Candan et al., 2009). SEM pictures of each taxon were taken at magnifications of 200x, 500x, and 1000x to record their microstructural traits.

2.1.1.1 Choosing an Operational Taxnomic Units (OTU's)

Operational Taxonomic Units (OTUs) are chosen as the first stage in a numerical taxonomic investigation. OTUs might be individuals, populations, species, or other types of organisms. The OTUs used in this study are species from the Plumbaginaceae genera found in Turkey. 61 OTUs (species) in all were utilized. In this, 61 OTUs were examined in all, and they are all members of the *Acantholimon* (Plumbaginacea) genus.

2.1.1.2 Character Selection and Coding of those Characters

This phase involves choosing the characters, followed by coding them. Use a vast range of characters, and try to avoid having any of them overlap. It makes more sense to select a character from all of the OTU's. Characters often combine qualitative and quantitative traits. States of a character might be contradictory or diverse. Depending on the character, It is advisable to select as many characters as you can because it is thought that higher character counts would result in more accurate correlations across OTUs. The characters we used here are morphological and have been taken from all of the otu's.

Table 2-1 Characters Table

Bract characters:		
	Bract number:	Average number
	Outer bract length (mm):	mm
	Outer bracket shape:	Ovate, Suborbiculate(0) Ovate,Lanceolate (1) Onling-linear, Lanceolate (2) Ovate Ovate,triangular (3) Oblong Lanceolate (4) , Triangular lanceolate (5), Lanceolate (6) , Lanceolate-obivate (7), Ovate (8), Oblong-triangular (9), Subovate triangular (10), Triangular (11), Linear-lanceolate (12), Ovate-triangular (13), Oblong ovate (14)
	Inner bract length:	mm
	Inner bract shape:	Obovate (0), Oblong- elliptical (1), Oblong-lanceolate (2), Elliptical-lanceolate (3), Oblong-triangular (4), Lanceolate (5), Ovate-lanceolate (6), Oblong-ellipitical (8)
	Outer bract from the inner bract is:	Long(0), Short (1), Equal (2), Short and Equal (3), Short and Long (4), Long and Equal (5)
	External bract surface eg. Type:	Regualr Sulcate (0), Irregular Sulcate (1), Irregular Sinuate (2)
	Outer bract Number of rows of cells with unit area:	Average

Table 2-1: Characters Table (Continued)

External bracte cell surface structure:	Smooth-Striate (0), Striate-Reticulate (1)
Outer bract hair present are:	_ (0), +(1)
Outer bract hair type is:	_ (0), Puberulent(1), Pubesent (2),Puberulent-Pubesent (3), Vilos (4), Lanatus (5), Stigillous (6)
Hair size (µm):	µm, - (0)
Number of hairs per unit area of the outer bract:	Average
Outer bract hairs on the costa :	_(0), +(1)
External bract stoma type:	Anisocytic (0), Anemocytic (1), Anisocytic-Anemocytic (2)
External bract Stoma length:	µm
Number of stomata per unit area of the outer bract:	Average
Outer bract Cell dimensions (µm)	µm
Spik Characteristics	
Spike height :	cm
Spiklete height:	mm
Florat number:	Average
Caylx characteristic	
Calyx height:	mm
Calyx lip length :	mm

Table 2-1: Characters Table (Continued)

Calyx lip tip shape:	Ttuncate (0), Acute (1), Obtuse (2), Ovate (3), Turncate-Obtuse (4), Acute-Obtuse (5), Triangle-Obtuse (6), Acute-Cuspidate (7)
Calyx veins are:	Short (0), Long (1), Short or Long(2), Extend to the Edge (3), Extend to the Edge Short (4), Extend to the Edge long (5).
Calyx surface epidermal cell wall type:	Regular (0), Sinuate (1), Reticulate (2), Regular Sinuate (3), Irregular Sinuate (4), Regular-Sinuate- Reticulate (5), Irregular-Sinuate-Reticulate (6),
Number of cell rows per calyx unit area	Average
Calyx epidermal margins	Acute –(0), + (1), Obtuse –(0), +(1), Truncate –(0), + (1)
Caylx stoma is:	Not Present –(0), Present +1(1)
Number of stomata per unit area:	Number , -(0) means not present
Calyx hairs:	-(0) means not Present, +(1) means present
Calyx tube in diameter hairs are:	-(0) means not Present, +(1) means present.
hairs scattered :	-(0), messy(1), On the vein(2), between the vein (3)
calyx tube size:	mm
Calyx tube hairs are:	-(0) means hair are not Present, +(1) hairs are means present.

Table 2-1: Characters Table (Continued)

Hairs scattered /on the veins/between the veins:	-(0), Messy(1), between the vein(2), On the vein(3)
calyx tube in diameter type:	-(0), Lanatus(1), Pilos(2), Puberlant(3), Velutinus(4), Vilos(5)
hair size:	µm
Number of hairs per unit area:	Average
Number of stomata per unit area:	Average

2.1.1.3 Construction of Data Matrix

Quantitative and qualitative data were the two categories of data we used. nonetheless, we categorize the data that is qualitative as binary states like 0 or 1 for the existence and absence of character traits. Because there are unique patterns within genera that allow for the distinct delimitation of groups having specified numbers of flowers, bracts, etc., quantitative features enumerated by number of flowers, spikelets, bracts, etc. have been coded. Arithmetic mean is used to express measurements like size, dimension, and ratios. These types of data are written as numerical values within a 0.01 scale rather than being converted to binary states.

CHAPTER 3

RESULT AND DISCUSSION

32 Characteristics of 61 taxa ware examined in this study with SEM. We examined the qualitative and quanititave characteristics of 61 taxa, which have been shown in the pages below (all of these SEM figures have been retrieved from (Candan et al., 2009). These characteristics are important for the classification of the taxa

3.1 Micromorphological Characters

In this study, the micro morphological traits of the studied *Acantholimon* taxa—flower, flower throat, flower tube, and bracts—calyx were evaluated. A variation was clearly seen in terms of the structures, and the data were grouped into three branches as trichomes, stomas, and surface sculpturing pattern are given below.

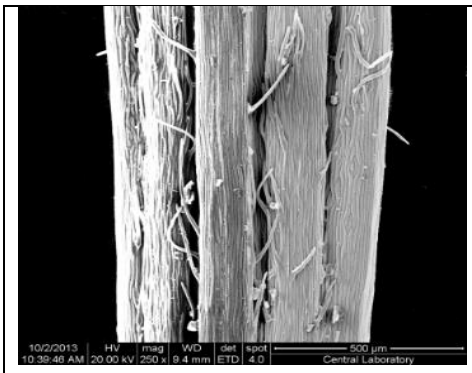
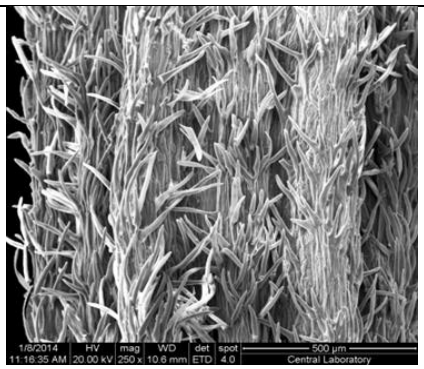
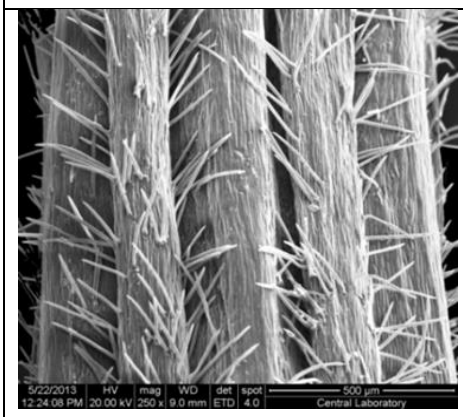
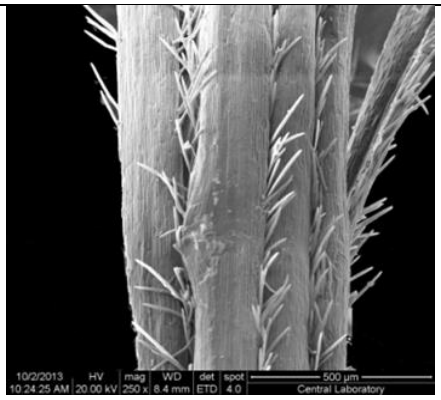
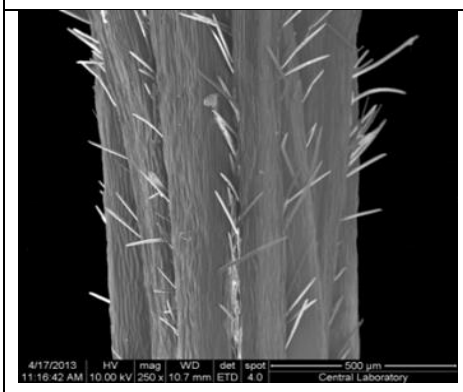
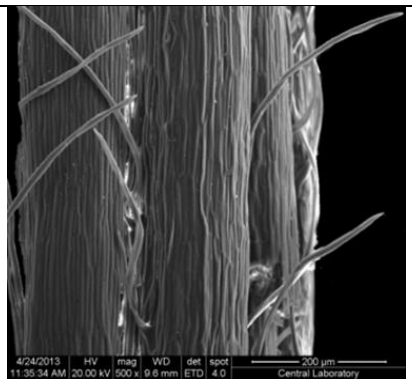
3.1.1 Trichomes (Trichomes on the flower throat and calyx tube)

Except for *A. bashkaleicum*, *Acantholimon* possesses shorter, erratic, and many trichomes (Figure 6). *Sec.Staticopsis,* on the other hand, has longer, more uniformly arranged hairs that are located on the veins. On the basis of their hairs, taxa with hairs on the flower neck and calyx tube may be divided into three categories.

a)Trichomes messy

b) Trichomes on the veins

c) Trichomes between the veins

	
A. bashkaleicum	Trichomes messy, *A. capitatum subsp. sivasicum*
	
A. huetii var. *huetii*	*A. köycegizicum*
	
A. puberulum subsp. *puberulum*	Fig 6b.Trichomes on the veins, *A. acerosum* subsp. *acerosum* var.

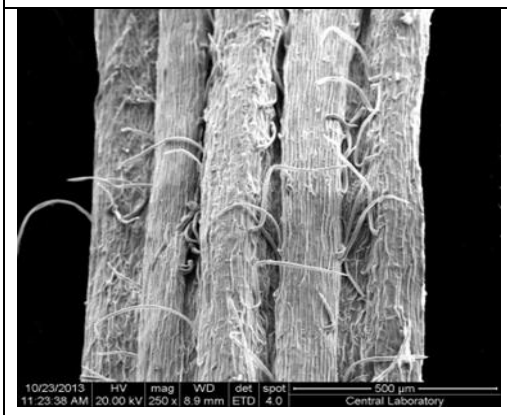
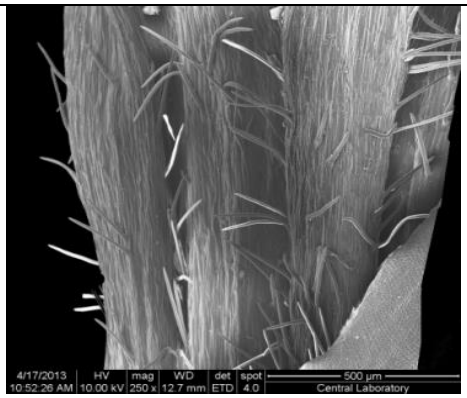
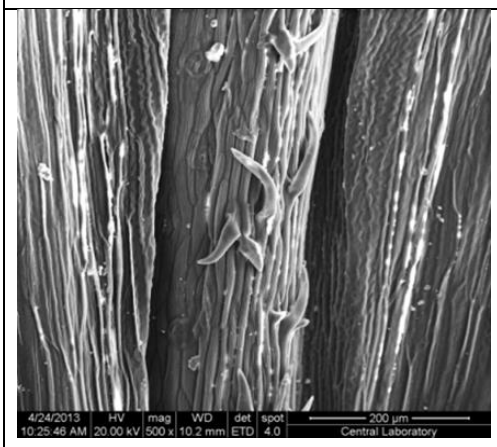
	parvifolium
	
A. birandii	Trichomes between the veins, *A. puberulum* subsp. *longiscapum*
	
A. hohenackerii	

Figure 3-1: *A. Bashkaleicum*, , *A. acerosum* subsp. *acerosum* var. *parvifolium* , *A. puberulum* subsp. *longiscapum*

3.1.1.1 Trichomes on the outer bracte

It has been noted that certain species of the *Acantholimon* genus are distinguished by having hairs on the outer bracts. All members of the *Acantholimon* section and all members of the *Tragacanthina* section lack hairs on their outer bracts, with the exception of *A. bracteatum*, which has the least hairs (1 hair) per unit area. Additionally, the remaining members of the *Robustea*, *Dianthifolium*, and *Caryophyllacea* subsections of the Staticopsis section do not have hairs on their outer bracts, with the exception of the taxa *A. glumaceum*, *A. spirizianum,* and A. venestum var. venestum. Additionally, every member of the *Circinnatea* subsection has hairs on its outer bracts, with the exception of A. hypochaerum.

The following trichome forms are found in the genus:

- strigillous
- pubescent
- puberulent
- lanatus
- vilous
- pilose

The dominant taxon, A. bracteatum, shows strigillous-type tiny hairs. The bracts of Sect. Staticopsis mostly have long, irregularly shaped hairs. The bracts of all taxa, with the exception of *A. spirizianum*, are covered with trichomes(Figure 7). Interestingly, *A. spirizianum* differs from other species by having trichomes exclusively on the costal portions of the bracts(Figure 7a).

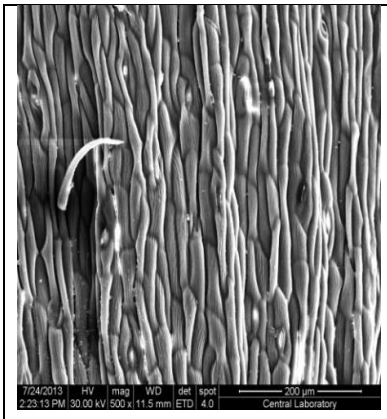
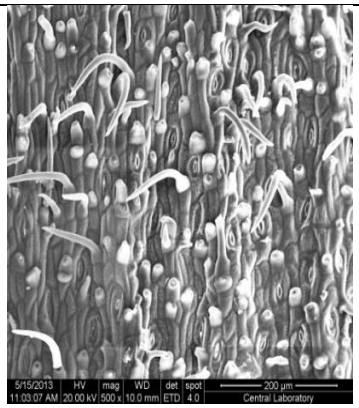
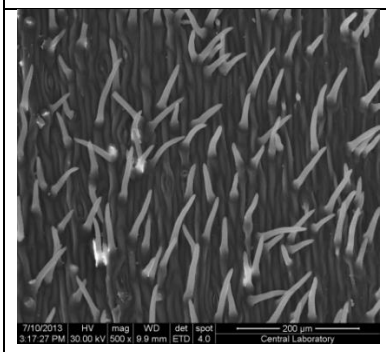
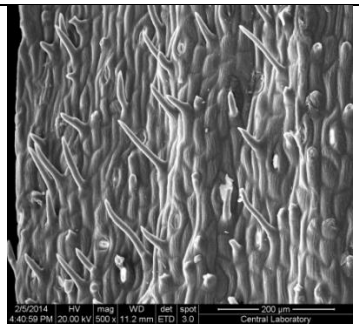
	

Figure 7a.Strigilous, *A. bracteatum*	*A. strigillosum*
	
A. calvertii var. calvertii	*A. göksunicum*

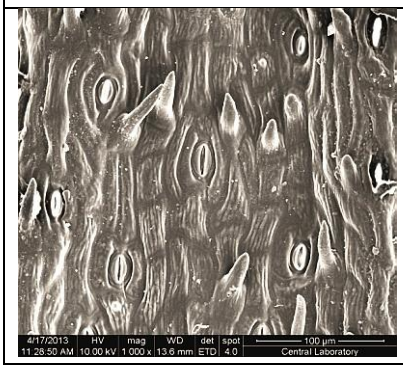



Figure 7b.Puberulent *A. puberulum* subsp. *peroninii*	*A. venustum* var. *assyriacum*

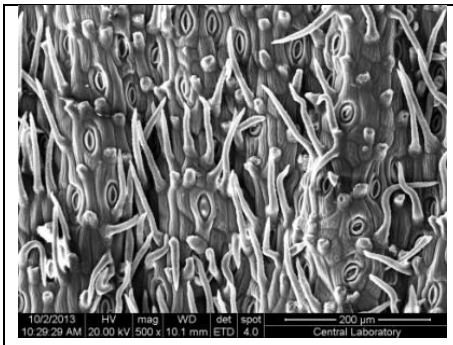
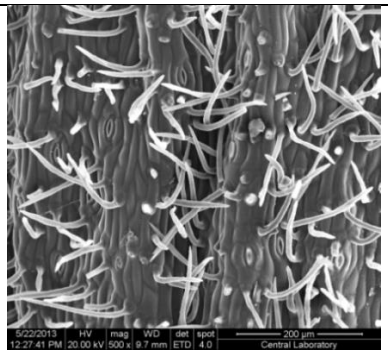
	

Figure 7c. Pubescent *A. köycegizicum*	*A. huetii* var. *huetii*

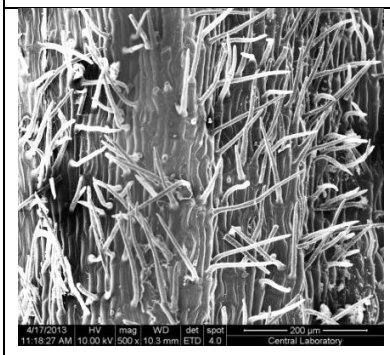
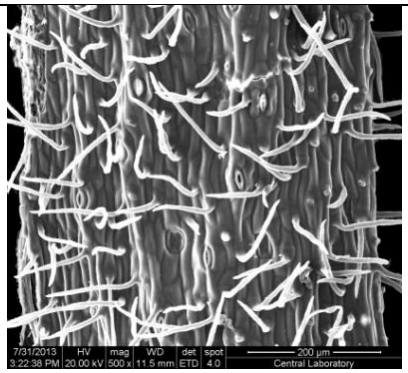

	

Figure 7d. Vilos, A. *puberulum* subsp. *puberulum*	*A. anatolicum*

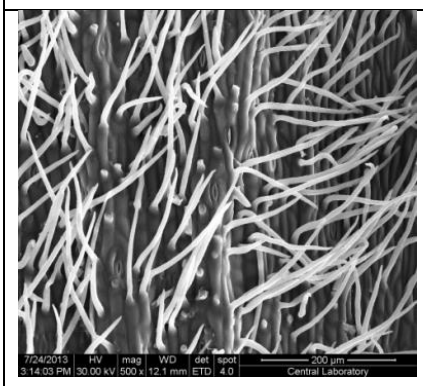
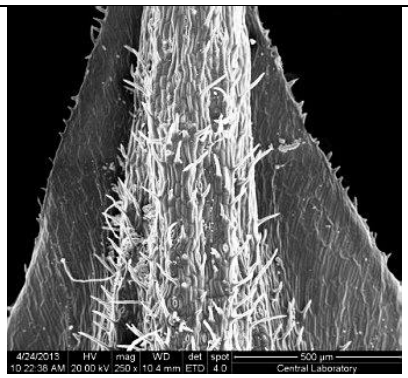

	

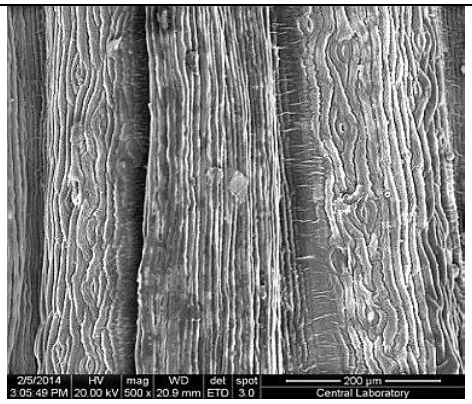
Figure 7e.Lanatus *A. ulicinum* var. *creticum*	Figure 7f.*A. spirizianum*, trichomas on the costa

Figure 3-2: *A. Bracteatum, A. puberulum* subsp*. Peroninii, A. Köycegizicum,* A. *puberulum* subsp. *Puberulum, A. ulicinum* var. *Creticum, A. spirizianum*

3.1.1.2 Stoma

Stomata may be observed in the calyx only in the species *A. quinquelobum*, which belongs to the *Tragacanthina* division (Figure. 8a). The stomata types present on the flower neck, calyx tube, and bracts exhibit the same characteristic in all taxa. Stomata are not seen in the sinuate areas between the veins; they are only seen on the veins with sulcate ornamentation on the flower neck and calyx tube (Figure 8b).

Figure 8a.Caylx in stoma, *A quinquelobum var. quinquelobum*



A *quinquelobum* var. *curviflorum*

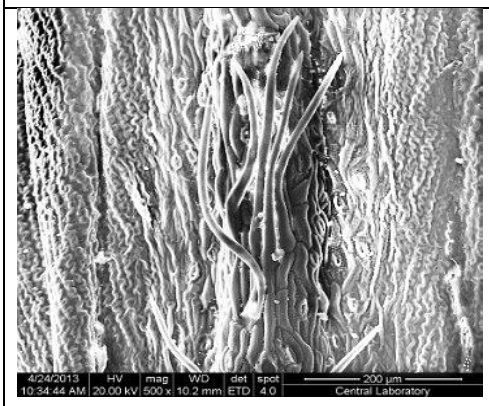
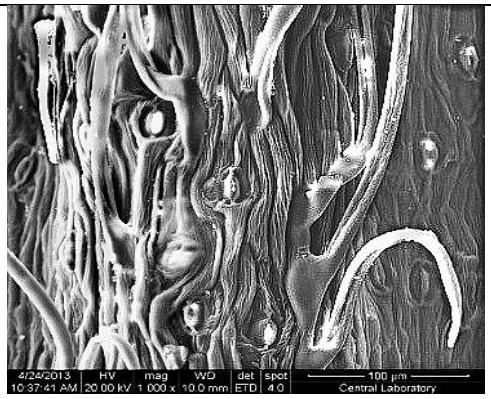


Figure 8b stoma in calyx tube, sinuate-sulcate structure, *A. confertiflorum*



Close view of stomas and trichomes

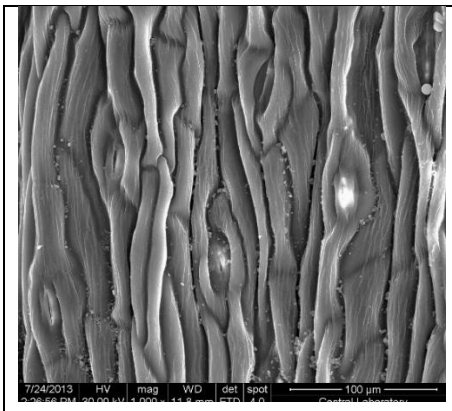
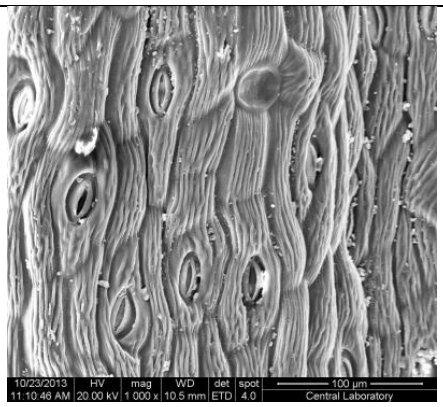
	
A. bracteatum	*A. karamanicum*

Figure 3-3: *A quinquelobum var. Quinquelobum, A. confertiflorum*

The genus can be divided into three groups based on the varieties of stomata present on the flower neck, calyx tube, and outer bracts. Either group has both anisocytic and anemocytic stomata, or it has solely anisocytic stomata and anemocytic stomata.

All species of the genus contain stomata on the outer bracts. *A. bracteatum and A. bashkalicum* have been two species in the *Acantholimon* section that have anisocytic-type stomata, while *A. strigillosum, A. ulicinum,* and *A. karamanicum* are three species in the Subsection *Androsacea,* and *A. acerosum, A. damassanum, A. hoheneckerii, A. saxifragiforme,* and *A. Venestum* in the subsection *Caryophyllacea* have stomata of the anisocytic type (Figure 9a).

The majority of species in this genus have anisocytic-anemocytic stomata (Figure 9b), however a small number of species, such as *A. capitatum* from the section *acanthlimon* and *A. laxiflorum* from the section *Robustea,* have anemocytic stomata, which is very interesting (Figure 9c).

Furthermore, only the species *A. spirizianum* possesses calyx costae with stomata (Figure. 9d), while in the species, some parts of the bract (nearly 2/3) have stomata (Figure. 9e).

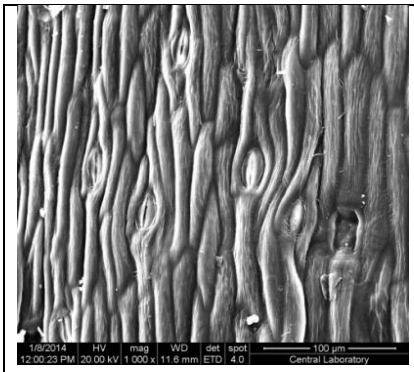
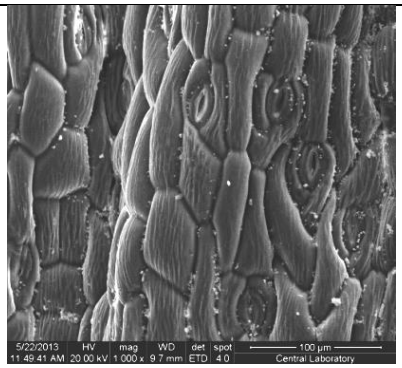
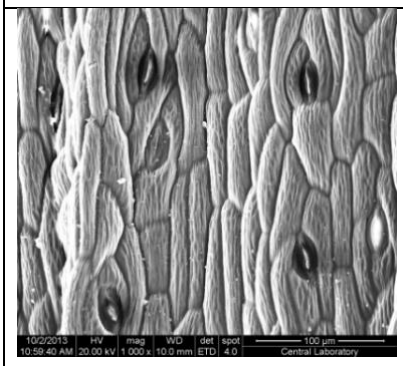
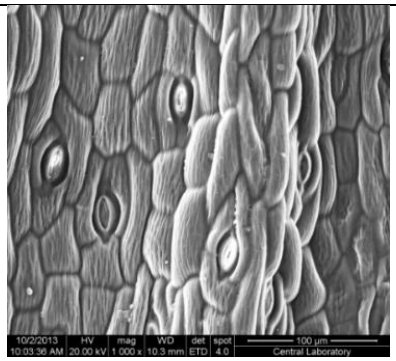
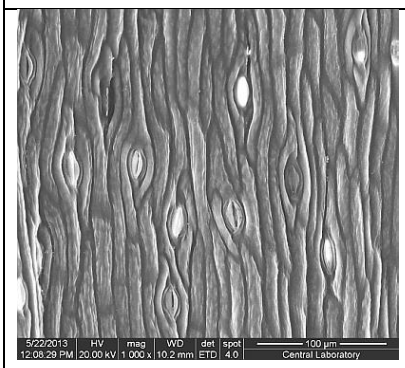
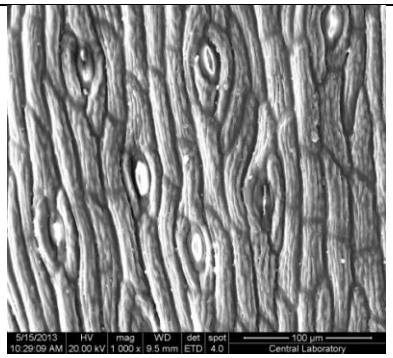
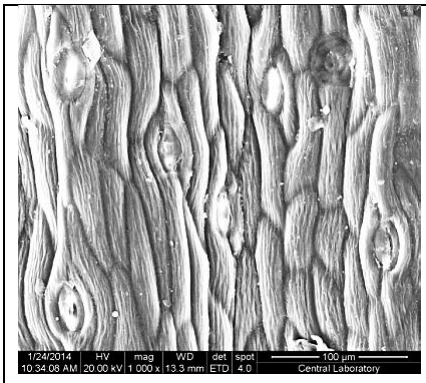
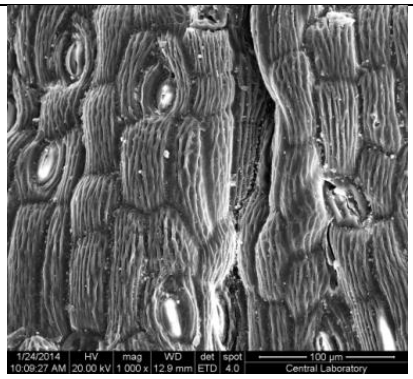
	

Figure 9a.with only anisositic stoma, *A. acerosum* subsp. *acerosum*	*A. saxifragiforme*
	
A. hoshapicum	*A. artosense*
	
A. hypochaerum	*A. caesareum*

	
A. petraeum	*A. senganense*

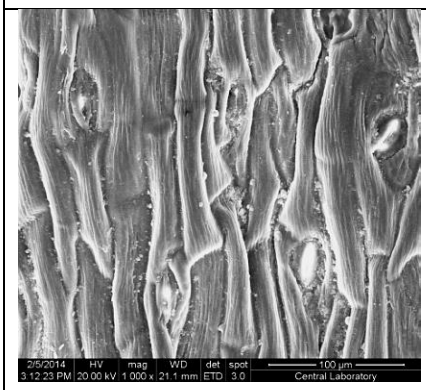
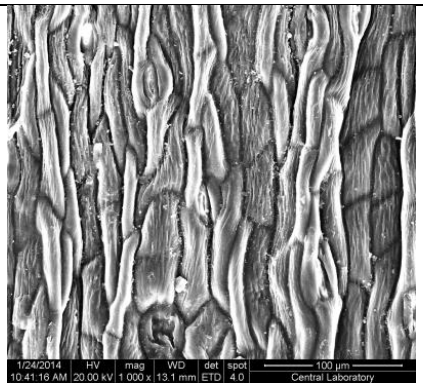

	

Figure 9b.with anisositic-anemositic stoma *A. lepturoides*	*A. hohenackerii*

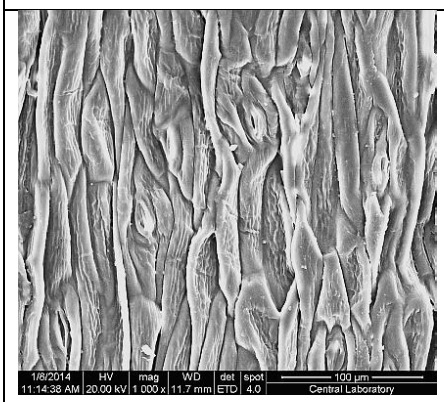
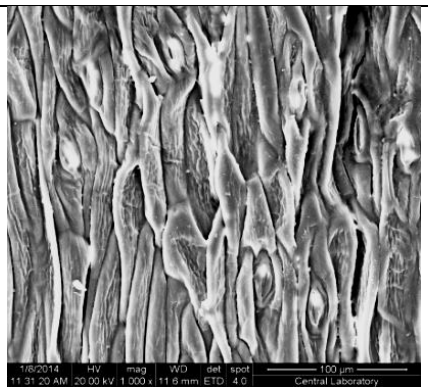

	

Figure 9c.with anemositic stoma *A. capitatum subsp. sivasicum*	*A. laxiflorum*

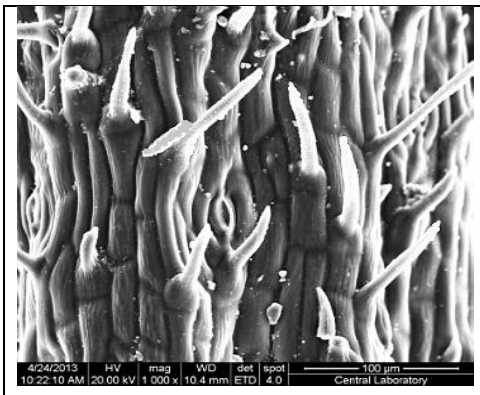
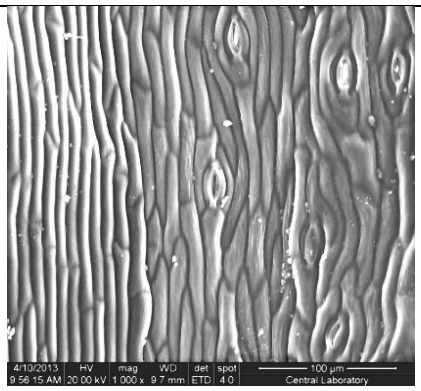
	

Figure 9d. *A. spirizianum*, close view of the stomas on the costa	Figure 9e. *A. multiflorum*, some part of the bract have stomas

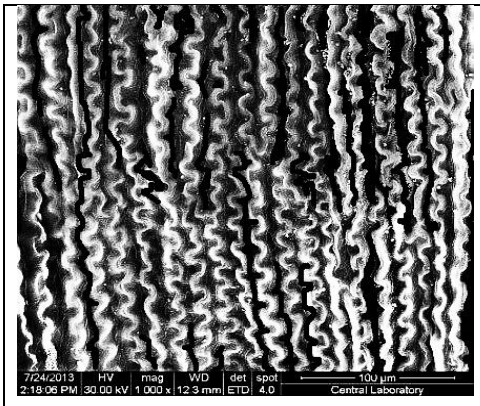
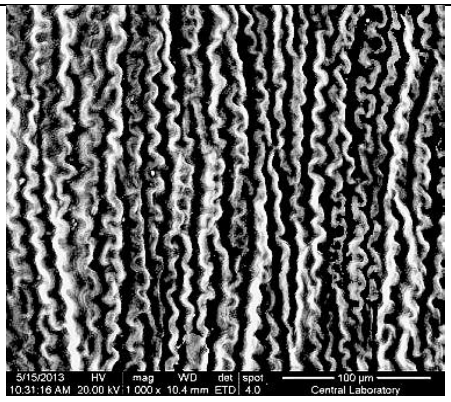
	
A bracteatum(regular sinuate pattern)	*A caryophyllaceum*

Figure 3-4: *A. acerosum* subsp. *Acerosum, A. Lepturoides, A. capitatum subsp. Sivasicum, A. Spirizianum, A. Multiflorum.*

3.1.1.3 Surfaces Sculpturing Pattern

Acantholimon flowers have six different styles of surface sculpture, which are listed below.

- Sinuate pattern
- Sinuate-reticulate pattern
- Reticulate pattern
- Sinuate-sulcate pattern
- Sulcate pattern
- Bract cell surface pattern

- **Sinuate pattern**

Sinuate pattern is divided into two subtypes:

Regular sinuate pattern (Subtype 1)

Irregular sinuate pattern (Subtype 2)

In the section *Aconthlimon*, regular (*A. bracteatum*) and irregular (*A. bashkeleicum)* patterns were discovered. However, only regular sinate patterns, such as those of *A. caryophllaceum*, have been seen in the subsection *Caryophylaceae*, whereas in the subsection *Circinnatea*, only irregular sinuate patterns (*A. Köycegizicum*) have been seen (Figure 10a)

- **Sinuate-reticulate pattern**

This pattern is not seen in the calyx; within Sec. *Acantholimon*, *Tragacanthina*, *Robustea,* and *Dianthifolia*. However, *A. puberulum* from Subsec. *Andracea* and *A. halophyllum* and *A. glumaceum* from Subsec. Caryophyllaceae serve as excellent examples of species that often display this pattern (Figure 10b).

- **Reticulate pattern**

Only two non-endemic species within Subsec. *Caryophyllaceae*, *A. hoheneckerii* and *A. fominii*, belonging to this genus, have calyx of this pattern type (Figure 10c)

- **Sinuate-sulcate pattern**

In all taxa, the calyx tube members exhibit the sinuate-sulcate pattern feature. The areas with vasculature, the costal parts, show a sulcate pattern, while the intercostal areas where there is no vasculature exhibit a sinuate pattern.Within the genus, only the calyx of the species *A. quinquelobum* has the sinuate-sulcate feature. Furthermore, the presence of stomata in the sulcate region is an important taxonomic characteristic for the genus (Figure 10d).

- **Sulcate pattern**

Sulcate surface ornamentation is seen on the bracts of the *Acantholimon* genus. While the surface decorations of the outer bract is more prominent and so desirable for inspections, both the inner and outer bracts possess the same trait. To further understand the bract sulcate pattern of the members of the *Acantholimon* section, two subtypes should be considered.

Sulcate pattern that is regular (Subtype 1)

Sulcate pattern that is erratic (Subtype 2)

Species like *A. hypochaerum, A. calvertii, and A. ekimii* have a consistent sulcate characteristic in Subsec. *Circinnate* (Figure. 10e). On the other hand, every

member of Sec. *Tragacanthina* as well as the majority of the members of Sec. Staticopsis exhibit irregular sulcate ornamentation (Figure 10f).

Two types of irregular ornamentation are seen within cells:

Taxa with distinct cell boundaries (Figure 10g)

Taxa with indistinct cell boundaries (Observed only in 2 taxa within the genus, *A. capitatum*).

- **Bract cell surface pattern**

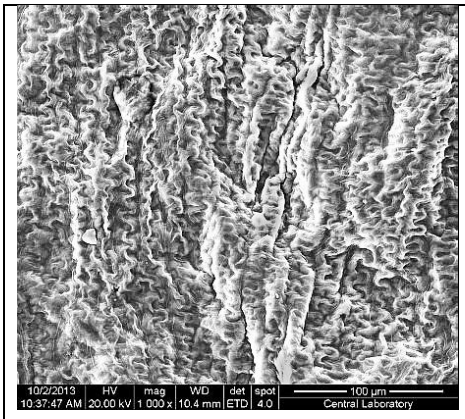
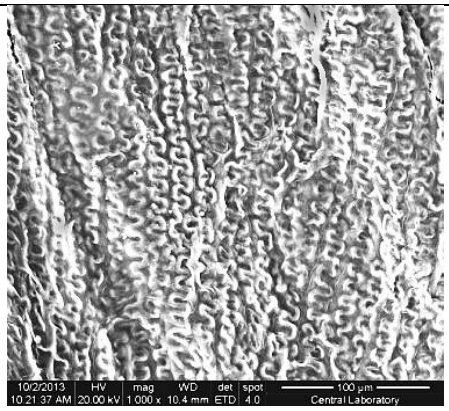
There are two kinds of the *Acantholimon* genus' bract cell surface ornamentation:

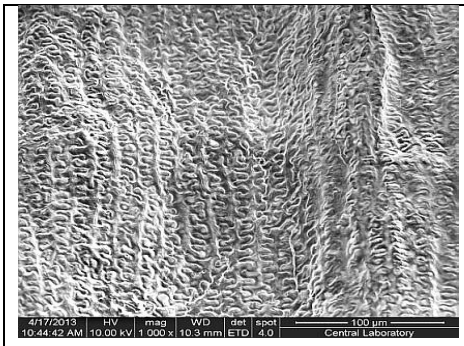
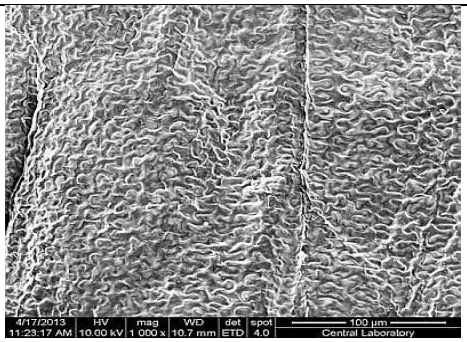
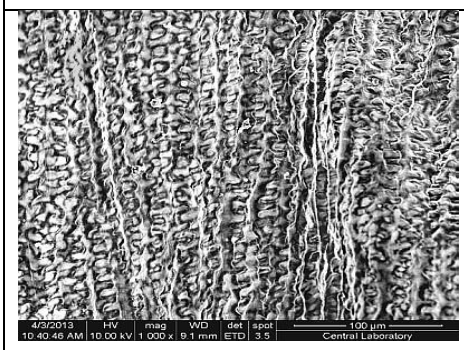
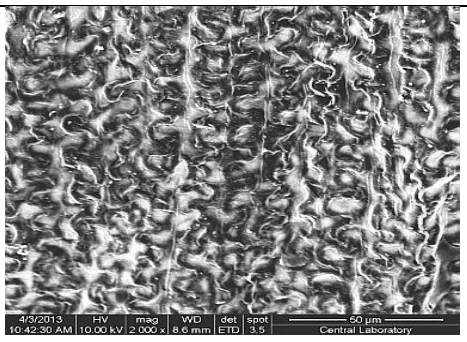
(Subtype 1) Smooth-striate

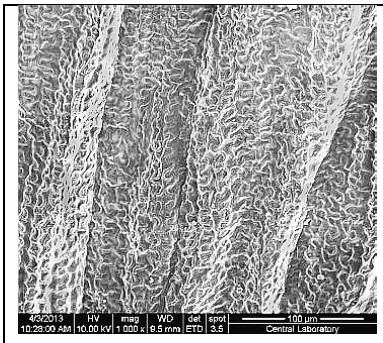
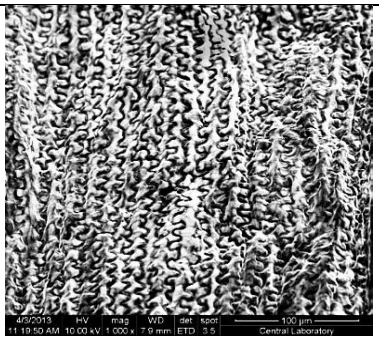
(Subtype 2) Striate-reticulate

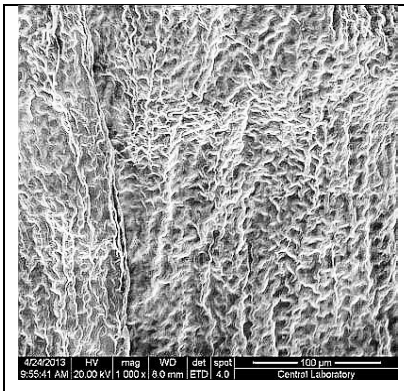
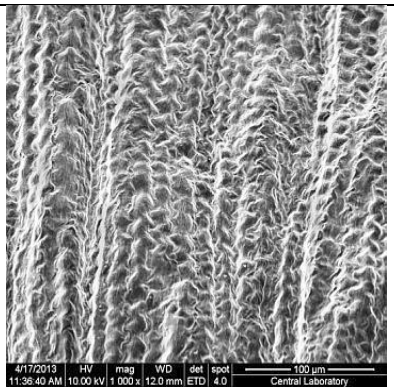
When it comes to bract cells, species such as A. *bracteatum and A. petuniiflorum* coming from Sec. *Acantholimon; A. laxiflorum* from Subsec. *Robustea*; *A. hypochaerum* and *A. calvertii* from Sec. *Circinnetea*; and *A. ekimii, A. anatolicum, A. strigillosum, and A. uliicinum* from Subsec. *Andrasacea* present among the most distinct examples of the smooth-striate subtype (Figure 10h).

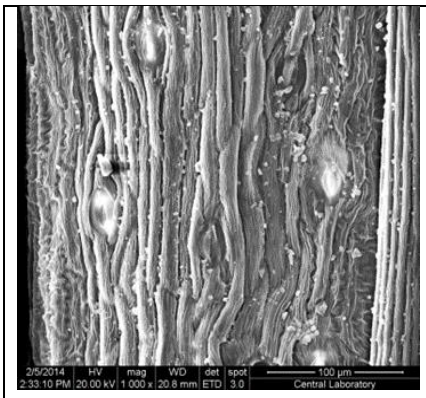
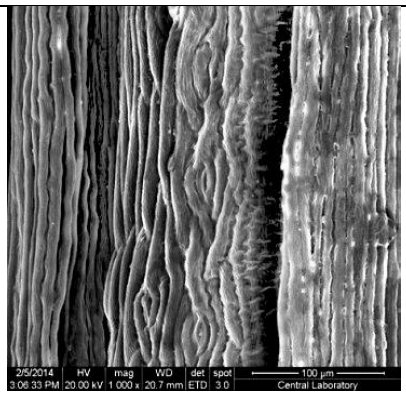
However, just the species *A. hohenackerii* displays the smooth-striate characteristic in bract cells within Subsec. *Caryophyllacea*. The bract cells of all other species in the genus exhibit striate-reticulate ornamentation (Figure. 10i).

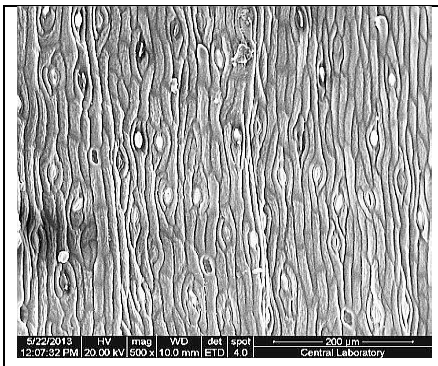
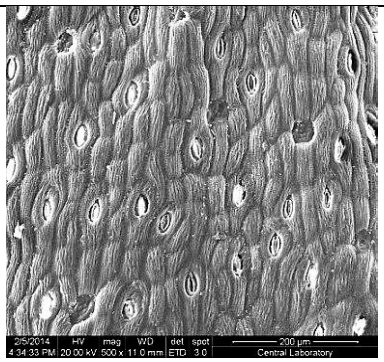
	
A. bahscaleicum	*A. köycegizicum*
Figure 10 a. Irregular sinuate pattern(Subtype2)	

	
A. puberulum subsp. longiscapum	*A. puber subsp. Peronii*
	
A. reflexifolium	*A. halophilum , halophylum*

	
A. halophilum var. coloratum	*A. glumaceum*
Figurer 10b. Sinuate-Reticulate Pattern (Type 2)	

	
A. hohenackerii	*A. fominii*
Figure10c. Reticulate pattern (Type 3)	

	
A. quinquelobum var. quinquelobum(close view of calyx)	*A. quinquelobum var. curviflorum* (close view of calyx)
Figure 10d. Sinuate-Sulcate Pattern (Type 4)	

	
A. hypochaerum	*A. armenum* var. *Armenum*

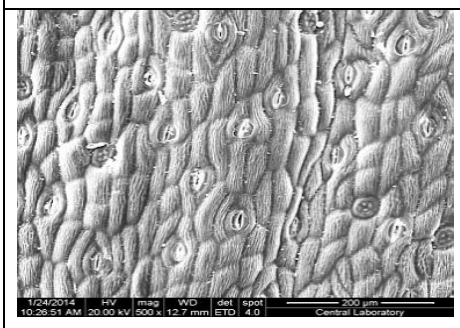
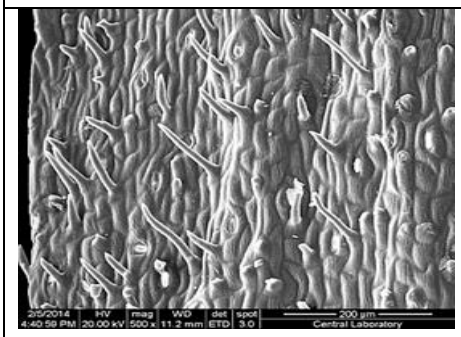
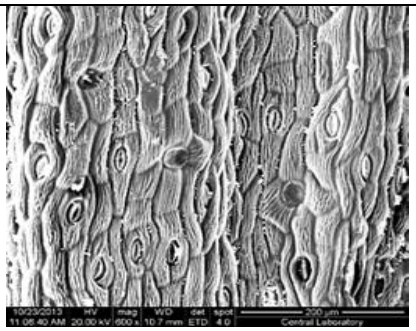
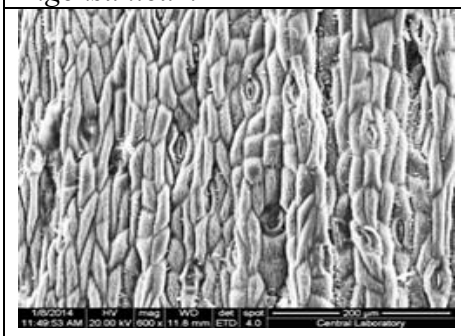
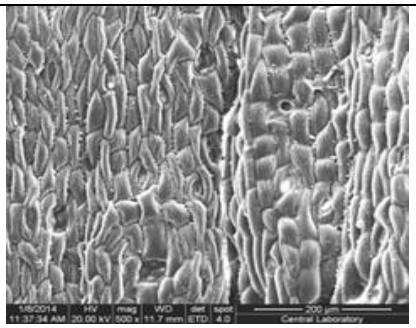
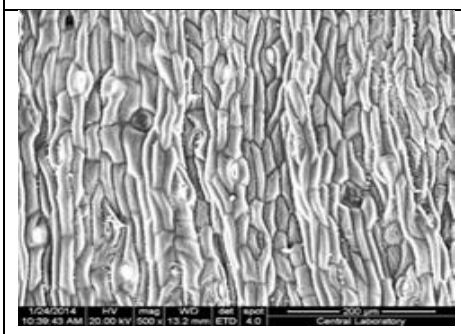
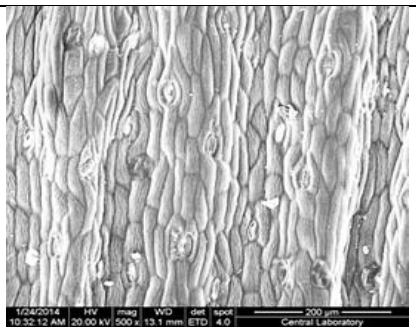



Figure 10e. outer bract surface ornemantation, reguler sulcate, *A.*	*A.quinquelobum var quinquelobum*

libanoticum	
	
A.göksunicum	*A. karamanicum*
	
A. iconicum	*A. damassanum*
	
A. hoheneckerii	*A. petraeum* (Figure 10f. outer bract surface ornemantation, irreguler sulcate)

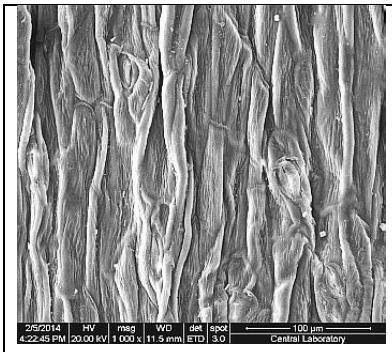
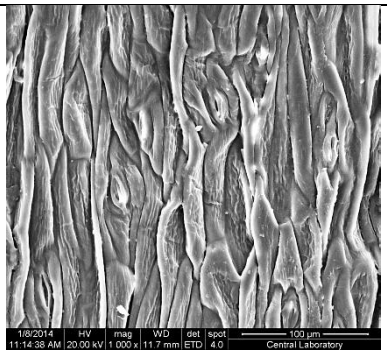
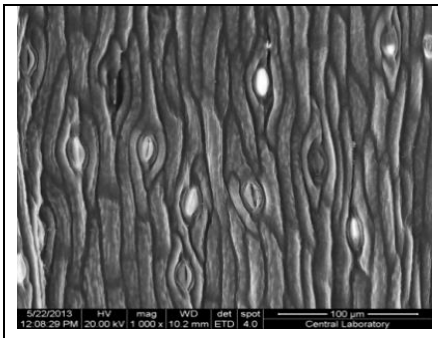
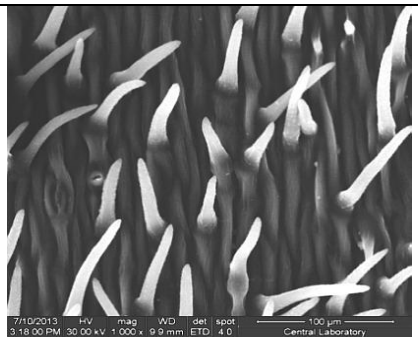
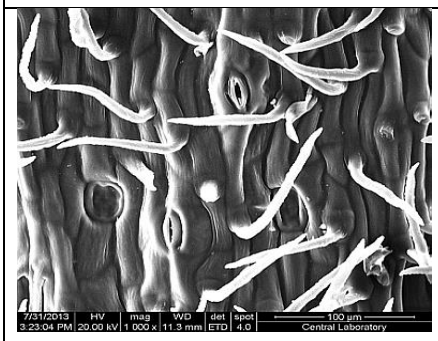
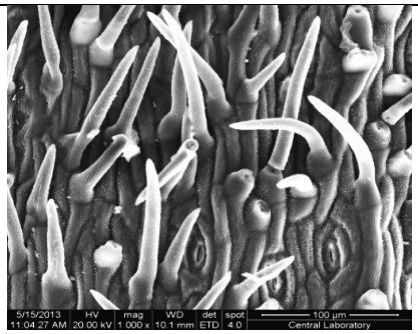
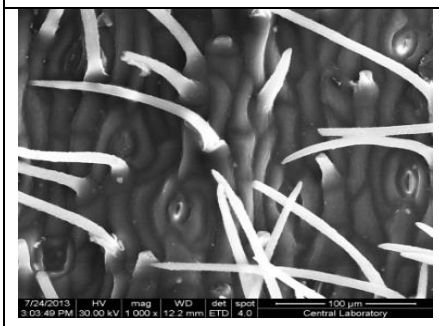
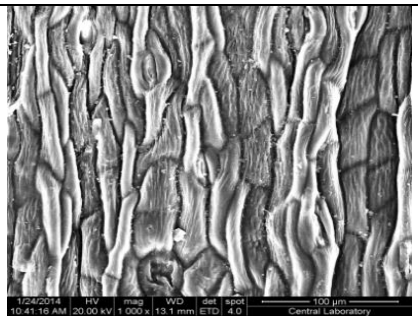
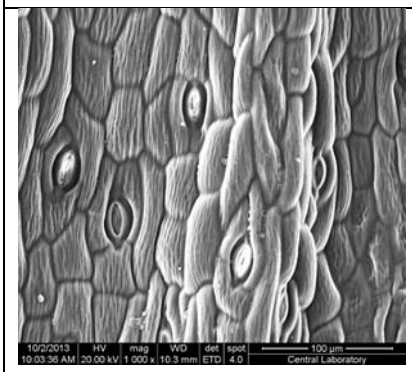
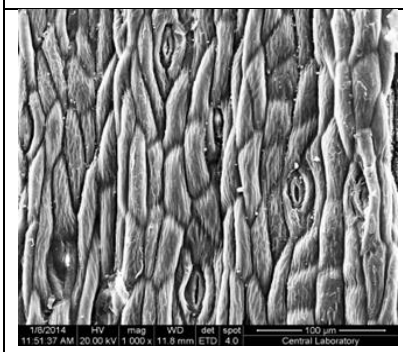
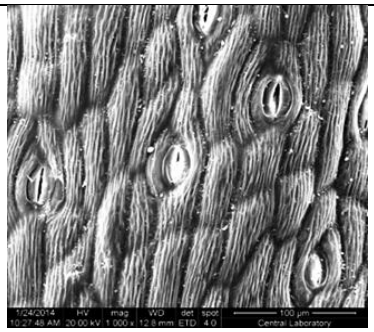
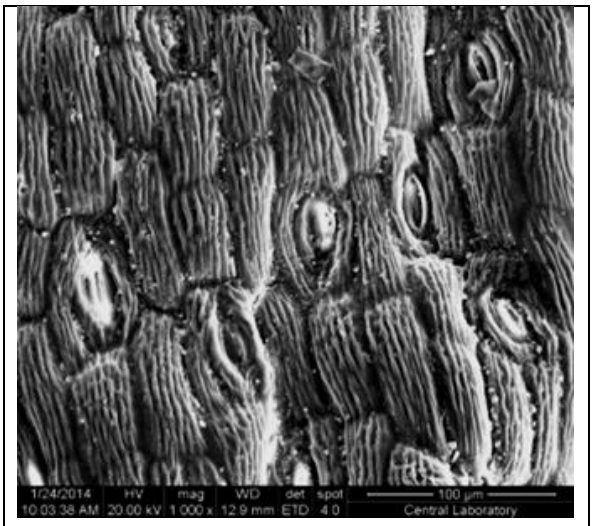
	

Figure.10g, *A.capitatum* subsp. *capitatum*	*A.capitatum* subsp. *Sivasicum*

	
A. hypochaerum	*A. calvertii* var. *calvertii* Figure.10h.outer bract cell surface structure,a)smooth-striate (subtype 1),
	
A. anatolicum outer bract cell surface structure,a)smooth-striate (subtype 1),	*A. strigillosum* outer bract cell surface structure,a)smooth-striate

	(subtype 1),
	
outer bract cell surface structure,a)smooth-striate (subtype 1), *A. ulicinum* var. *ulicinum*	*A. hohenackerii* (Figure.10i.outer bract cell surface structure,b)striate-reticulate (subtype 2)
	
A. artosense	*A. köycegizicum*
	
A. birandii	*A. libanoticum* (.outer bract cell surface structure,b)striate-reticulate (subtype 2)



A. parviflorum

Figure 3-5: *A. Bahscaleicum, A. halophilum var. Coloratum, A. Hohenackerii, A. quinquelobum var. Quinquelobum, A. Libanoticum, A. Petraeum, A.capitatum* subsp. *Capitatum, A. ulicinum* var. *ulicinum*

3.2 Cluster Analysis results

The results of the cluster analysis carried out by the mvsp program with the Gower similarity coefficient can be seen in the figure 11 as a UPGMA phenogram; a tree had been separated into two subgroups by two lines that were drawn. In figure 11, we drew a line at 0.85 and another line at 0.88. These lines separate the trees into several clusters since it is obvious that certain species from the same subsection are distant from one another. They are close to some other species in the cluster. According to this study, morphological traits are therefore helpful for identification to some extent, but we cannot completely rely on that.

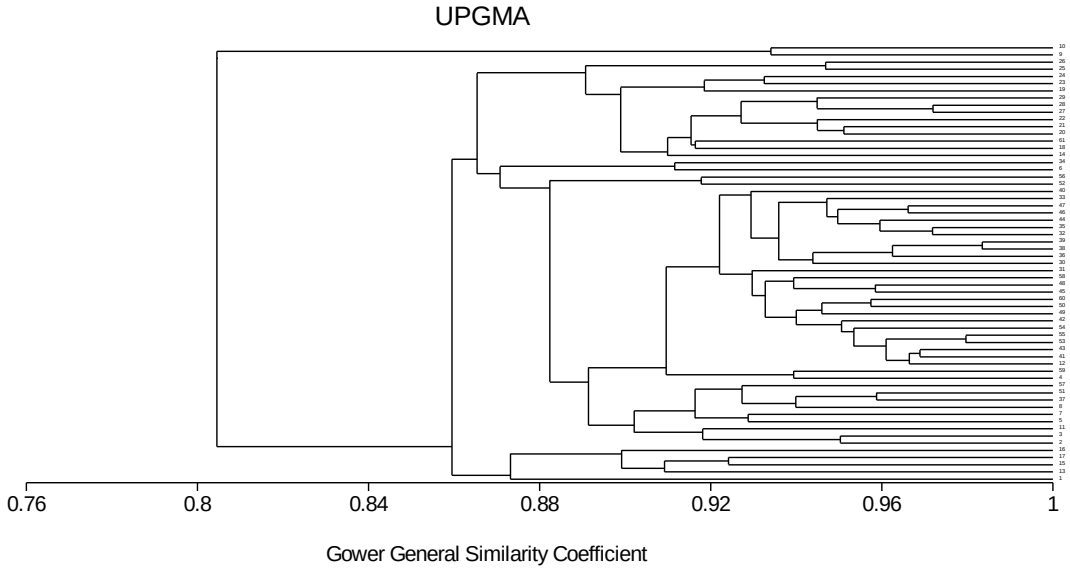


Figure 3-6: UPGMA phenogram: Plumbaginaceae in Turkey

The list of species in Figure 11 is ordered by their ordinal numbers.

1. *A. bracteatum*
2. *A. capitatum subsp. Capitatum*
3. *A. capitatum subsp. Sivasicum*
4. *A. hoshapicum*
5. *A. bashkaleicum*
6. *A. artosense*
7. *A. petuniiflorum*
8. *A. tragacanthinum*
9. *A. quinquelobum var. quinquelobum*
10. *A. quinquelobum var. Curviflorum*
11. *A. laxiflorum*
12. *A. dianthifolium*
13. *A. Hypochaerum*

14. A. köycegizicum

15. A. calvertii var. Calvertii

16. A. calvertii var. glabrum

17. A. ekimii

18. A. göksunicum

19. A. huetii var. Huetii

20. A. huetii var. Breviscapum

21. A. wiedemannii

22. A. yildizelicum

23. A. anatolicum

24. A. strigillosum

25. A. ulicinum var. ulicinum

26. A. ulicinum var. creticum

27. A. puberulum subsp. Puberulum

28. A. puberulum subsp. Longiscapum

29. A. puberulum subsp. Peroninii

30. A. karamanicum

31. A. birandii

32. A. confertiflorum

33. A. reflexifolium

34. A. caesareum

35. A. caryophyllaceum

36. A. acerosum subsp. acerosum var. acerosum

37. A. acerosum subsp. acerosum var. parvifolium

38. A. acerosum subsp. Brachystachyum

39. A. acerosum subsp. longibracteolorum

40. A. avanosicum

41. A. armenum var. armenum

42. A. armenum var. balansae

43. A. lepturoides

44. A. kotschyi

45. A. iconicum

46. A. halophilum var. halophilum

47. A. halophilum var. coloratum

48. A. lycaonicum subsp. Lycaonicu

49. A. damassanum

50. A. hohenackerii

51. A. saxifragiforme

52. A. glumaceum

53. A. libanoticum

54. A. parviflorum

55. A. senganense

56. A. spirizianum

57. A. araxanum

58. A. fominii

59. A. petraeum

60. A. venustum var. venustum

61. A. venustum var. assyriacum

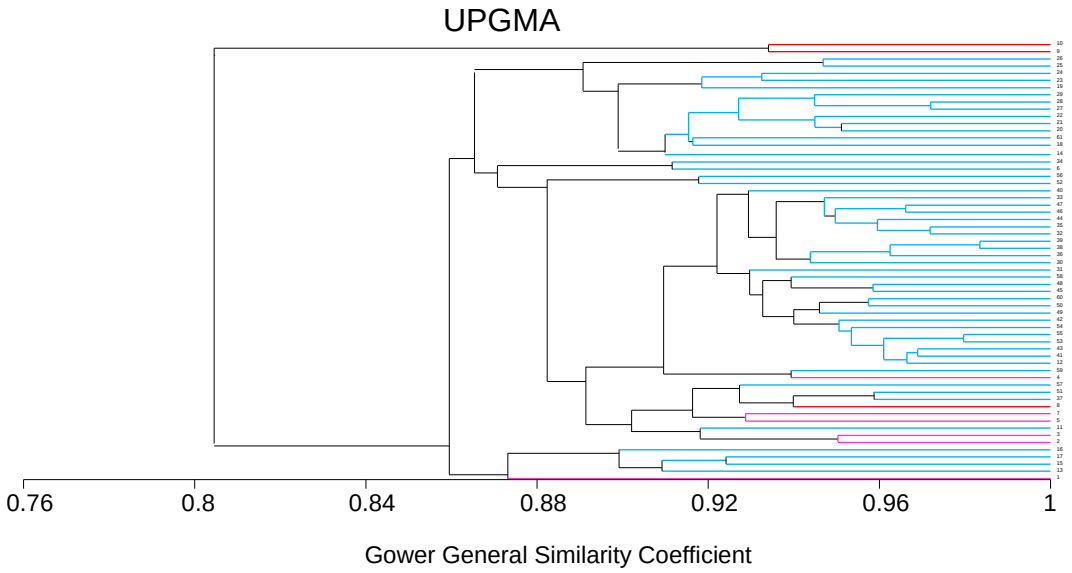


Figure 3-7: UPGMA phenogram: Plumbaginaceae in Turkey

We can observe that *A. bracteatum* is not close to their cluster on our phenogram diagram. *Acontholimon* species lack hairs on their outer bracts, but the *A. bracteatum* possesses one hair per unit, which may explain why it is located distant from the cluster. Only a few species, including *A. glumaceum, A. spirizianum*, and *A. venestum var. venestum*, have hairs on the outer bracts in the *Dianthifolium* and *Caryophyllacea* subsections of the Staticopsis section. With the exception of the taxonomy hypochaerum, however, the hairs on the outer bract of subsection *Circinnatea* are visible. This may be the cause of some of these taxa's proximity to one another. because *A. venestum var. venestum* near the *Circinnatea* is clearly seen in our diagram.

The taxon A. quinquelobum is the only one with stomata on its calyx. This taxon belongs to the Tragacanthina sect. This might be the reason for the gap in our figure between the taxa A. quinquelobum and A. tragacanthinum. Furthermore, the sinuate-sulcate feature is found only in the calyx of the species A. quinquelobum within the genus.

The bract surface of this taxon, *A. laxiflorum*, is smooth-striate. It is unusual among *Acontholimon* species. However, this taxon possesses anemocytic stomata, which are uncommon in the genus *Acontholimon*. Anemocytic stomata are found in just a few species, such as *A. capitatum* from the *Acantholimon* section. In our phenogram, we can observe that *A. laxiflorum* is separated from their cluster and is located near *A. capitatum*.

CHAPTER 4

CONCLUSIONS

Micromorphology research is essential for comprehending the micromorphological traits of the species and is also crucial for classifying various species. When it comes to of its micromorphological characteristics, the genus Plumbaginceae is quite complicated. Because of this, its micromorphological characteristics and their phylogenetic implications have not been extensively researched globally. Scanning electron microscopy (SEM) was used to analyze the micromorphology of 61 species of Acantholimon for this reason. And cluster analysis were also used. We looked at many micromorphological features, including , calyxes, bracts, and hairs. We examined qualitative and quantitative traits, such as hair size, the length of micromorphological features, etc.

We examined qualitative and quantitative traits, such as hair size, the length of micromorphological characteristics, etc., and a clear variance was seen. But due to such variations, we divided our findings into three groups. trichomes, stomata, and surface sculpuring patterns are these three groupings.

If we discuss the trichomes in this category, they are short and disorganized. Long trichomes are often organized and are usually found on the vein. We can categorize the trichomes in this taxon into three groups. Tricomes, the first group, are short and messy The second group includes lengthy, present trichomes on the vein, and the third includes trichomes discovered between the vein.

Trichomes were also discovered on the outer bract, a characteristic of acantholimon that is uncommon. Only a few species such as *A Dianthifolium A. glumaceum, A. spirizianum,* and *A. venestum var. venestum,* have hairs on the outer bract.This

particular trait is not present in the other spices in the genus. Additionally, the types of trichomes found in the group are listed below:

strigillous vilos pilos, lanatus, pubescent, and puberulent.

The study's other significant result was that only 2 *A. Tragacanthinum* and *A. quinquelobum* species of this genus have stomata on the calyx; the remaining species have stomata on the calyx tube and on the bract. It is divided into three categories depending on the types of stomata that are found on the calyx tube, the bract, and the calyx. The *Acantholimon*'s six surface sculpture patterns were also visible. Here are those examples. Bract cell surface pattern, Sinuate pattern, Sinuate-reticulate pattern, Reticulate pattern, Sinuate-sulcate pattern. Sect. *Acantholimon*, Sect. *Tragacanthinaand, and Sect. Staticopisis* are the names of the three sections that make up Acantholimon. Five more subsections make up Sect. Staticopisis. Clusters were observed through phenogram analysis. But different species from different parts are not located near to one another. Consequently, micromorphological traits are somewhat helpful for identification.

Acantholimon is divided into three sections, which are called *Acantholimon, Tragacanthinum,* and *Staticopsis.*

- **Sect. *Acantholimon***

Hetrophyllous leaves with prolonged spring leaves are present. Condensed spike-filled inflorescence capitate. It has 4-6 bracteate infundibular calyxes and 2-5 flowers.

A.bracteatum, A. capitatum, A. artosense, A. bashkalecicum, A. hoshapicum,and A. petuniiflorum are the species found in this section.

We can observe that *A. bracteatum* is not close to their cluster on our phenogram diagram. All *Acontholimon* species lack hairs on their outer bracts, but the *A.*

bracteatum possesses one hair per unit, which may explain why it is located distant from the cluster.

- **Sect. *Tragacanthina***

The plants in this division have tubular calyxes and heterophyllous leaves. They have one flower and three bracteates. *A. quinquelobum* (including curviflorum) along with *A. tragacanthinum* are included in this section.

Stomata on the calyx have only been observed in the taxon *A. quinquelobum*. This taxon is a member of the *sec. Tragacanthina*. This may be the cause of the distance between taxa *A. quinquelobum* and *A. tragacanthinum* in our figure. Furthermore Only the calyx of the species *A. quinquelobum* in the genus exhibits the sinuate-sulcate characteristic.

- **Sect. *Staticopisis***

The species in this group have leaves that are monomorphic. Spikelets are 2-ranked, 1-flowered, 3-bracteate, simple or branching inflorescences, and the calyx is infundibular like an acanthliomon segment. This section is divided into five more subsections.

Only a few species, including *A. glumaceum, A. spirizianum,* and *A. venestum var. venestum,* have hairs on the outer bracts in the *Dianthifolium and Caryophyllacea* subsections of the *Staticopsis* section. With the exception of the taxonomy hypochaerum, however, the hairs on the outer bract of subsection Circinnatea are visible. This may be the cause of some of these taxa's proximity to one another. because A. venestum var. venestum near the Circinnatea is clearly seen in our diagram.

- **Subsect. *Robustea***

A. laxiflorum Boiss is only one species found in this subsection. It has long, surpassing leaves that range in length from 18 to 38 cm, spikelets that range in number from 16 to 36, measure 13 to 15 mm, and bracteate 1-3. Calyx limb, purple-brown. This taxon, *A. laxiflorum*, exhibits a smooth-striate subtype of bract surface. It is uncommon among other species of acontholimon.

- **Subsect. *Diantifoliea***

Approximately 1.5 to 5 cm long, scapes are comparable in length to leaves. significantly distichous spikes in the inflorescence. The length is typically 12 to 15mm, and the number of spikelets ranges from 6 to 12. There are white and light pink calyx limbs visible. *A. dianthifolium* is the name of the species found in this subsection.

- **Subsect. *Crinnatea***

The leaves in this category are quite lengthy. 2-5 branching thick spikes of inflorescence. The length of the spikelets ranges from 11 to 17mm, and their number ranges from 4 to 16. Brownish-purple occasionally with a white calyx limb. *A. hypochaerum, A. koeyceizicum, A. calvertii, A. ekimii, A. goeksunicum, and A. huetii* are all present in this section.

- **Subdect. *Androsacea***

Inflorescence a single, short-branched spike with tightly clustered spikelets at the tip that range in length from 12 to 14 mm.They have Calyx limbs that are white. *A. lycaonicum, A. wiedemannii, A. ulicinum, A. puberulum, A. karamanicum, A. birandii, and A. confertiflorum* are the species that are present in this subsection. This section's A. puberulum is the greatest exhibit of the Sinuate-reticulate pattern.

- **Subsect. *Caryophyllacea***

Simple, generally slack, two-ranked spikes make up the inflorescence. This subsection's calyx limb is white and contains the species *A turcicum, A reflexifolium, A caryophyllaceum, A parviflorum, A acerosum, A avanosicum, A caesareum, A lepturoides, A kotschyi, A armenum, A araxanum, A libanoticum, A iconicum, A. fominii, A halophilum, A. senganense, A. spirizianum, A. petraeum and A. Venustum* (Do et al., 2007). Excellent examples of taxa that frequently exhibit this Sinuate-reticulate pattern include *A. glumaceum* from *Subsec. Caryophyllaceae.*

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APPENDICES

DATA MATRIX

The data matrix shown in Table C1 was utilized to create a phenogram. Characters from Table 3 can be seen in the first row, and species are presented in the first column**.**

Table 5-1: Data Matrix

	ch.1	ch .2	ch. 3	ch.4	ch. 5	ch.6	ch. 7	ch. 8	ch. 9	ch. 10	ch. 11	ch. 12	ch. 13	ch. 14	ch . 15	ch. 16
1	0	22	0	1	6	123.5	1.5	0	0	38.5	5	7.1	28.2	0	0	3
2	1	37	1	0	0	0	0	0	2	52	5	156	315	0	2	3
3	1	31	1	0	0	0	0	0	2	56.5	6	10.5	21.0	0	2	4
4	0	29	1	0	0	0	0	0	2	63.5	6	9.8	24.5	5	1	3
5	0	28	1	0	0	0	0	0	0	60.5	1.5	68	14.0	2	3	4
6	0	27	0	0	0	0	0	0	0	54.5	7	108	35.2	7	3	3
7	0	17	1	0	0	0	0	0	2	51	4	69.8	31.5	1	1	4
8	1	21	1	0	0	0	0	0	2	44.5	4	11.1	30.2	2	3	3
9	1	29	1	0	0	0	0	0	2	39.5	10	6.7	34.9	6	3	7
10	1	23	1	0	0	0	0	0	2	44	10	6.6	36.6	6	3	7
11	2	29	1	0	0	0	0	0	1	44.5	6	6.9	28.0	N/A?	3	3
12	1	19	1	0	0	0	0	0	2	59.5	8	7.0	30.5	2	3	3
13	0	27	0	0	0	0	0	0	2	45	11	6.8	17.7	N/A	1	5
14	1	25	1	1	1	85	32.5	0	1	41.5	9	10.0	28.7	N/A	3	4
15	0	21	0	1	1	87.5	32.5	0	2	43.5	1.5	6.9	16.9	N/A	3	3
16	0	37	0	1	1	41	1.5	0	2	32.5	8	3.5	17.0	N/A	3	3
17	0	19	0	1	1	90.5	9	0	2	38	1.5	11.1	28.9	N/A	1	5
18	1	19	1	1	6	82.5	11	0	2	31	5	5.0	27.1	N/A	3	3
19	1	19	1	1	2	101	25	0	2	47.5	5	6.5	31.5	3	3	2
20	1	25	1	1	2	109	12.5	0	2	32.5	5	5.2	14.4	3	3	3
21	1	27	1	1	3	55.5	25	0	2	33	3	9.5	28.0	6	3	5
22	1	25	1	1	3	56.5	12.5	0	2	47.5	5	7.3	22.1	4	0	3
23	1	19	0	1	4	82.5	15	0	2	50	2	10.0	28.0	N/A	3	3
24	1	21	0	1	6	71	25	0	1	35.5	3	6.8	20.5	0	3	3

Table 5-1: Data Matrix (Continued)

	ch.1	ch.2	ch.3	ch.4	ch.5	ch.6	ch.7	ch.8	ch.9	ch.10	ch.11	ch.12	ch.13	ch.14	ch.15	ch.16
25	1	17	0	2	1	11.5	15		0	31.5	3	7.0	21.0	N/A	1	6
26	1	19	0	1	5	120	26	0	0	37.5	3	7.1	25.5	N/A	1	6
27	1	23	1	1	4	111.5	15	0	1	35.5	5	6.8	24.0	N/A	3	6
28	1	23	1	1	5	151	15	0	0	31.5	6	7	8	N/A	3	6
29	1	31	1	1	1	40.5	7.5	0	2	32.5	7	7.3	20.5	N/A	3	6
30	1	21	1	0	0	0	0	0	0	40	11	10.5	31.8	N/A	3	4
31	1	19	1	0	0	0	0	0	2	42	5	6.6	21.6	N/A	3	3
32	1	17	1	0	0	0	0	0	2	49	7	5.0	20.8	N/A	3	3
33	1	17	1	0	0	0	0	0	2	46.5	5	6.9	28.8	2	1	6
34	0	21	1	0	0	0	0	0		55.5	7	6.5	18.0	N/A	3	3
35	1	17	1	0	0	0	0	0	1	42	10	6.7	24.7	N/A	3	3
36	1	19	1	0	0	0	0	0	0	44	7	6.7	24.6	N/A	0	6
37	1	21	1	0	0	0	0	0	0	41.5	6	6.7	18.5	N/A	0	4
38	1	19	1	0	0	0	0	0	0	48	7	9.8	28.5	N/A	0	3
39	1	19	1	0	0	0	0	0	0	46.5	7	10.2	33.0	N/A	0	6
40	1	21	1	0	0	0	0	0	1	60	7	6.8	27.8	2	3	3
41	1	19	1	0	0	0	0	0	1	57.5	6	10.1	32.0	N/A	3	3
42	1	15	1	0	0	0	0	0	1	64	9	7.0	29.8	N/A	3	4
43	1	13	1	0	0	0	0	0	1	63	4	7.1	31.0	0	3	3
44	1	15	1	0	0	0	0	0	1	58	8	7.0	28.0	3	3	3
45	1	23	1	0	0	0	0	0	2	51.5	6	9.9	26.7	3	3	2
46	1	25	1	0	0	0	0	0	2	43	6	10.3	27.8	1	3	3
47	1	21	1	0	0	0	0	0	2	45.5	4	55	45.5	1	3	3
48	1	17	1	0	0	0	0	0	1	42	6	10.1	25.0	5	3	2

Table 5-1: Data Matrix (Continued)

	ch.1	ch. 2	ch. 3	ch. 4	ch .5	ch. 6	ch.7	ch .8	ch. 9	ch. 10	ch. 11	ch. 12	ch. 13	ch. 14	ch. 15	ch. 16
49	1	17	1	0	0	0	0	0	0	50	4	9.7	27.6	0	3	6
50	1	19	1	0	0	0	0		0	55.5	3	9.5	34.0	N/A	3	2
51	1	15	1	0	0	0	0	0	0	58	6	6.6	42.0	N/A	1	6
52	1	25	1	1	1	44.5	15	0	1	39	4	5.3	19.0	N/A	5	3
53	1	13	1	0	0	0	0	0	2	59	5	9.4	41.6	N/A	1	3
54	1	13	1	0	0	0	0	0	1	62	5	9.2	35.0	3	4	6
55	1	9	1	0	0	0	0	0	1	61.5	5	9.6	30.0	0	1	3
56	1	17	1	1	2	63.5	15	1	1	36	2	7.0	27.6	3	3	3
57	0	21	1	0	0	0	0	0	2	42	7	9.7	27.0	N/A	N/A	6
58	1	15	1	0	0	0	0	0	1	51.5	5	4.9	27.2	N/A	3	2
59	1	19	1	0	0	0	0	0	2	50.5	6	7.0	27.0	N/A	1	3
60	1	29	1	0	0	0	0	0	0	51.5	4	10.1	34.0	N/A	3	3
61	1	19	1	1	1	68	1.5	0	0	39.5	4	10.0	24.8	N/A	3	2

Table 5-1: Data Matrix (Continued)

	ch. 17	ch. 18	ch. 19	ch. 20	ch. 21	ch. 22	ch. 23	ch. 24	ch. 25	ch. 26	ch. 27	ch. 28	ch. 29	ch. 30	ch.. 31	ch. 32
1	21	9	1	1	0	0	0	1	2	5.5	1	1	4	75	45	12.5
2	23	1	1	1	0	0	0	0	0	6	1	1	1	300	100	22.5
3	27	0	1	1	0	0	0	0	?	6	1	1	1	305	100	17.5
4	23	0	0	1	0	0	0	1	2	6.5	1	1	2	195	35	12.5
5	31	0	1	1	0	0	0	0	0	N/A	1	1	2	180	15	12.5
6	28	0	0	1	0	0	0	1	2	N/A	1	1	4	295	100	12.5
7	31	0	1	1	0	0	0	0	0	7.5	1	1	2	90	65	12.5
8	29	1	1	1	0	0	0	0	?	6.25	1	2	2	360	15	7.5
9	31	1	1	0	1	2	0	0	0	N/A	1	2	3	35	1.5	17.5
10	35	1	1	0	1	5.5	0	0	0	N/A	0	0	0	0	0	32.5
11	23	1	1	1	0	0	0	0	1	6.5	1	1	1	260	100	22.5
12	33	1	1	1	0	0	0	1	2	6.25	1	1	2	135	25	7.5
13	43	0	1	1	0	0	1	1	3	9.5	1	3	2	75	15	7.5
14	27	1	1	1	0	0	1	1	3	7.5	1	3	2	200	65	22.5
15	31	1	1	1	0	0	1	1	2	8.25	1	1	2	75	55	7.5
16	21	0	1	1	0	0	0	0	2	8.25	1	2	2	65	12.5	7.5
17	47	0	1	1	0	0	0	1	3	6.5	1	3	2	125	35	7.5
18	31	1	1	1	0	0	0	1	2	7.5	1	2	2	163	45	12.5
19	?	0	0	1	0	0	1	1	1	8.5	1	1	5	240	100	17.5
20	29	1	1	1	0	0	1	1	1	8.5	1	1	5	275	75	17.5
21	27	0	1	1	0	0	1	1	1	6.5	1	1	5	280	55	7.5
22	33	1	1	1	0	0	1	1	1	9.5	1	1	2	265	45	7.5
23	27	1	1	1	0	0	1	1	1	6	1	1	5	300	100	17.5
24	39	0	0	1	0	0	1	1	2	5.5	1	1	5	260	65	22.5

Table 5-1: Data Matrix (Continued)

	ch. 17	ch. 18	ch. 19	ch. 20	ch. 21	ch. 22	ch. 23	ch. 24	ch. 25	ch. 26	ch. 27	ch. 28	ch. 29	ch. 30	ch. 31	ch. 32
25	23	0	1	1	0	0	1	1	3	5.5	1	2	5	164	45	17.5
26	27	0	1	1	0	0	1	1	2	5.5	1	2	5	305	45	17.5
27	31	0	1	1	0	0	1	1	1	7.5	1	2	2	240	45	3
28	25	0	1	1	0	0	1	1	1	7.5	1	1	2	255	35	3
29	23	0	1	1	0	0	1	1	1	7.5	1	1	2	320	35	3
30	25	0	1	1	0	0	1	1	2	5.5	1	1	5	125	45	3
31	27	0	1	1	0	0	0	1	3	N/A	1	3	2	235	15	3
32	21	0	1	1	0	0	1	1	2	6.5	1	2	2	230	7.5	7.5
33	29	0	1	1	0	0	1	1	1	7.5	1	2	2	195	25	7.5
34	33	0	1	1	0	0	0	1	2	8.5	1	1	5	275	100	7.5
35	23	1	1	1	0	0	1	1	2	9	1	2	2	245	35	7.5
36	33	0	0	1	0	0	1	1	2	5.5	1	1	2	160	7.5	7.5
37	33	0	1	1	0	0	0	0	0	5.5	1	2	2	290	7.5	7.5
38	29	0	1	1	0	0	1	1	2	5.5	1	1	2	305	35	7.5
39	27	0	1	1	0	0	1	1	2	5.5	1	2	2	310	35	7.5
40	25	0	0	1	0	0	1	1	2	N/A	1	2	2	255	15	3
41	21	1	1	1	0	0	0	1	2	5.5	1	1	2	340	15	3
42	23	1	1	0	0	0	0	1	2	5.5	1	1	2	200	15	7.5
43	29	1	1	1	0	0	0	1	2	5.5	1	1	2	125	25	12.5
44	25	1	1	1	0	0	1	1	2	6	1	1	2	150	25	12.5
45	N/A	1	1	1	0	0	0	0	2	7	1	1	2	130	25	7.5
46	27	1	1	0	0	0	1	1	2	7	1	1	2	130	25	7.5
47	41	1	1	1	0	0	1	1	2	7	1	1	2	135	25	7.5
48	N/A	1	1	1	0	0	0	1	2	6.5	1	1	2	110	25	7.5

Table 5-1: Data Matrix (Continued)

	ch. 17	ch. 18	ch. 19	ch. 20	ch. 21	ch. 22	ch. 23	ch. 24	ch. 25	ch. 26	ch. 27	ch. 28	ch. 29	ch. 30	ch. 31	ch. 32
49	43	1	1	0	0	0	0	1	2	7.5	1	2	2	105	25	7.5
50	N/A	1	1	1	0	0	0	1	2	6.5	1	2	2	65	15	7.5
51	45	0	1	1	0	0	0	0	0	8	1	2	2	115	15	7.5
52	25	0	1	1	0	0	0	0	3	7.5	1	1	2	240	15	17.5
53	21	1	1	1	0	0	0	1	2	7.5	1	2	2	135	15	7.5
54	37	0	1	1	0	0	0	1	2	8	1	2	2	245	15	7.5
55	33	1	1	1	0	0	0	1	2	7.5	1	2	2	210	15	7.5
56	27	1	1	1	0	0	0	0	0	5.5	1	2	2	195	15	7.5
57	71	1	1	1	0	0	0	0	0	??	1	2	2	160	7.5	7.5
58	N/A	1	1	0	0	0	0	0	3	7.5	1	1	2	160	7.5	3
59	23	0	0	1	0	0	0	1	2	6.5	1	1	4	285	15	7.5
60	25	1	1	1	0	0	0	1	3	9.5	1	2	2	160	25	12.5
61		1	1	1	0	0	1	1	2	9.5	1	1	2	255	25	12.5