LEAF ANATOMY OF FIVE SPECIES OF *LIMONIUM* MILL. (PLUMBAGINACEAE)

ZEYNEP BAL

MAY 2011

LEAF ANATOMY OF FIVE SPECIES OF *LIMONIUM* MILL. (PLUMBAGINACEAE)

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

BY

ZEYNEP BAL

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

MAY 2011

Approval of the thesis:

LEAF ANATOMY OF FIVE SPECIES OF *LIMONIUM* MILL. (PLUMBAGINACEAE)

submitted by **ZEYNEP BAL** in partial fulfillment of the requirements for the degree of **Master of Science in Biology Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Musa Doğan
Head of Department, **Biological Sciences** _____

Prof. Dr. Musa Doğan
Supervisor, **Biology Department, METU** _____

Examining Committee Members:

Prof. Dr. Sevil Pehlivan
Biology Dept., Gazi University _____

Prof. Dr. Musa Doğan
Biology Dept., METU _____

Assoc. Prof. Dr. Galip Akaydın
Biology Edu. Dept., Hacettepe University _____

Prof. Dr. Osman Ketenoğlu
Head of Biology Dept., Ankara University _____

Assoc. Prof. Dr. Sertaç Önde
Biology Dept., METU _____

Date: _____

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 : Bal, Zeynep

Signature :

ABSTRACT

LEAF ANATOMY OF FIVE SPECIES OF *LIMONIUM* MILL. (PLUMBAGINACEAE)

Bal, Zeynep

M.Sc. Department of Biological Sciences

Supervisor: Prof. Dr. Musa Doğan

May 2011, 86 pages

In this study, leaf anatomies of five species of *Limonium* Mill. (*L. echioides, L. globuliferum, L. tamaricoides, L. anatolicum* and *L. Sinuatum*) are studied. Taca belonging to five different sections of the genus *Limonium* as; *L. echioides* of the section *Schizyhymenium*, *L. globuliferum* of the section *Sphaerostachys*, *L. tamaricoides* of the section *Limonium*, *L. anatolicum* of the section *Sarcophyllum* and *L. sinuatum* of the section *Pteroclados* are used in this study. Throughout the species studied, *L. tamaricoides* and *L. anatolicum* are endemic to Turkey.

In order to examine anatomy of leaves, the paraffin sectioning and hand cross sectioning methods are used. Avarage stomata length, width and number per 210.68µm x 263.27µm area of the leaves are examined and the stomata types due to epidermal cells are defined. Additionally, the vascular bundles, upper and lower epiderms of leaves of each species are defined.

It is determined that except the species *L. globuliferum* and *L. tamaricoides*, the stomata density per 210.68µm x 263.27µm area of the leaves are approximately same for upper and lower epidermis, but in these species, the stomata numbers at upper epidermis is higher than the stomata numbers in lower epidermis, which might be related with the altitude, microclimate and habitat of these two species. It is also found that except the shoot leaf of *L. sinuatum*, the phloem is closer to lower epiderm than xylem in vascular bundles. But in shoot leaf of *L. sinuatum*, the phloem circularly covers the xylem and makes a closed circle around.

Keywords: *Limonium echioides*, *Limonium globuliferum, Limonium tamaricoides, Limonium anatolicum, Limonium sinuatum*, leaf anatomy, stomata density, stomata length, stomata width.

ÖZ

LIMONIUM MILL (PLUMBAGINACEAE) CİNSİNE AİT BEŞ TÜRDE YAPRAK ANATOMİSİ ÇALIŞMASI

Bal, Zeynep

Yüksek Lisans, Biyolojik Bilimler Departmanı

Tez Yöneticisi: Prof. Dr. Musa Doğan

Mayıs 2011, 86 sayfa

Bu çalışmada *Plumbaginaceae* familyasına dahil olan *Limonium* cinsine ait *Limonium eichoides*, *Limonium globuliferum, Limonium tamaricoides*, *Limonium anatolicum* ve *Limonium sinuatum* türlerinin yaprak anatomileri çalışılmıştır. Çalışılan her taxon *Limonium* cinsine ait 5 farklı seksyona aittir. Bunlardan *L. echioides* seksyon *Schizyhymenium*'da, *L. globuliferum* seksyon *Sphaerostachys*'da, *L. tamaricoides* seksyon *Limonium*'da, *L. anatolicum* seksyon *Sarcophyllum*'da ve *L. sinuatum* da seksyon *Pteroclados*'dadır.

Yaprakları incelemek üzere, dokulardan el kesitleri ve parafin yöntemi ile mikrotom kesitleri alma yöntemleri uygulanmıştır. Yaprakların 210.68µm x 263.27µm yüzeylerinden ortalama stoma sayısı hesaplanmış, ayrıca stomaların uzunluk ve genişlik ölçümleri de kayıt edilmiştir. Bunlara ek olarak, her tür için, iletim demetleri, üst ve alt epidermisler tanımlanmıştır.

Çalışma sonucunda görülmüştür ki, *L. tamaricoides* ve *L. globuliferum* türleri dışında diğer türlerde yapraktaki 210.68µm x 263.27µm lik alan için, üst ve alt

epidermisteki stoma yoğunluğu yaklaşık olarak aynı olmakla beraber, *L. tamaricoides* ve *L. globuliferum* da 210.68µm x 263.27µm lik yaprak alanındaki stoma yoğunluğu üst epidermiste alt epidermiste olduğundan fazladır. Bu durum bu iki türün mikroiklimi, bulundukları yükseklik ve habitatları ile ilgili olabilir. Ayrıca, *L. sinuatum*'a ait sürgün yaprağından alınan kesitler dışındaki türlere ait kesitlerde soymuksu boruların odunsu borulara oranla, alt epidermise daha yakın olduğu gözlemlenmiştir. Ancak, *L. sinuatum*'a ait sürgün yaprağındaki kesitlerde, soymuksu boruların, odunsu boruların çevresinde dairesel bir yapıda bulunduğu ve odunsu boruları bir çember gibi çevrelediği gözükmektedir.

Anahtar Sözcükler: *Limonium echioides*, *Limonium globuliferum, Limonium tamaricoides, Limonium anatolicum, Limonium sinuatum*, yaprak anatomisi, stoma yoğunluğu, stoma boyu, stoma genişliği.

To my family

ACKNOWLEDGEMENTS

I am grateful and thankful to my supervisor, Prof. Dr. Musa Doğan, for his encouragement and support throughout the development of this thesis. Also I would like to thank him with the deepest appreciation of my heart for his patience to me and my excuses.

I would like to express my indebtedness to Assoc. Prof. Dr. Galip Akaydın for supplying the plant samples that were used in this study, as well as the photographs of flowers of the samples and for his permission of my studying with the plant samples.

I would like to thank to my thesis juri members, Prof. Dr. Sevil Pehlivan, Prof. Dr. Osman Ketenoğlu, Assoc. Prof. Dr. Galip Akaydın and Assoc. Prof. Dr. Sertaç Önde, for their critics and suggestions.

I would like to thank to Assoc. Prof. Dr. Sertaç Önde, for his encouragement throughout the this study. He is a great confidant as well as his being an awesome teacher.

Thanks to my lab mate and also my dearest instructor Özlem Mavi, for her help and toleration to me throughout my thesis. Also I am thankful to her for alturism, positive energy, encouregement and sharing all her knowledge with me.

Thanks to my family, Hatice – Yılmaz Bal for their encouregment, support and for their patience to all my moody habits. I am grateful to them for their infinite patience with me during the period of writing the thesis. It would be so hard to complete this study without feeling their support and love with me.

I also thank to my 'brother' Emre Aksoy for being himself only and being with me and supporting my studies incorporeally, no matter how far he is. Also I should thank him for the article support that he found in the library of Texas, A&M University.

Finally, I would like to thank Gamze Çıplak, Ceyhun Kayıhan, Bülent Ateş, Güven Öğüş, Burçin Öğütlü, Tuğçe Akkuş, Yıldırım Aydın, İlker Demiroğlu and Emre Akgöl for their understanding, encouragement, patience and friendship.

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LIST OF ABBREVIATIONS

CR	Critically Endangered
EN	Endangered Species
VU	Vulnerable to extinction
LC	Low Concern

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CHAPTER 1

INTRODUCTION

Plumbaginaceae Juss. is the sole family that is belonging to the ordo Plumbaginales in world wide (Jussieu , 1789). 24 Genera and 775 species are representing the family worldwide. The habitat of the family is mostly arid and saline soils. The species that are belonging to *Plumbaginaceae* family have medicinal, ecological and ornemental values (Heywood, 1978). In Turkey, the family *Plumbaginaceae* is represented by 6 genera as *Acantholimon* Boiss., *Plumbago* L., *Limonium* Miller, *Goniolimon* Boiss., *Limoniopsis* Lincz. and *Armeria* Willd (Davis 1982).

Some cytological, morphological and anatomical studies were made on the family *Plumbaginaceae*, especially on the genera *Acantholimon* Boiss. and *Limonium* Mill. are known (Doğan & Akaydın 2005; Akaydın 2007; Faraday & Thomson 1986; Saez, Carvalho & Resello 1988; Doğan & Akaydın 2002; Artelori 1989; Zhou & Song 2007; Doğan, Duman & Akaydın 2008).

Akaydın (2007) defined a new species *Limonium simithii* Doğan & Akaydın, which was an endemic to Irano-Turanian region and listed it in the CR category of the IUCN criteria due to its having a small and limited distribution in the salt steppe around Seyfe Gölü, Kırşehir at 1085 m. The new species flowers in June and grows with *Limonium globuliferum* (Boiss. & Heldr) O. Kuntze and *Limonium tamaricoides* Bokhari in the defined area. It was placed in the section *Limonium* due to its being perennial and having basal rosettes formed by entire leaves and having scapes with sterile branches and obconical calyces with 5-lobes.

A study related with a new species was done by Saez, Carvalho and Resello in 1988. They described a new species *Limonium leonardi – llorensii* by the revision of *Limonium marisolli L. llorens* from the coastal populations of South West Mallorca. They mentioned the several distinguishing morphological characters with the addition of the information about the different chromosome number of the new species from the endemic *L. marisolli* L. *llorens*. Several different habitat characters of these two species are defined. While the new species *L. leonardi – llorensii* grows up on the maritime slopes on calcarenite rocks of two South West Mjorcan localities, the old endemic species *L. marisolli* Gil & Llorens is restricted to a few coastal localities on South Western of the Palma Bay. They also pointed out that although both species have leaves with anisocytic stomata that are regularly distributed along the leaf blade, and the length of stomata guard cells are considerably shorter in *L. marisolli* than *L. leonardi – llorensii.*

In 2002, a study was done on the genus *Acantholimon* Boiss. by Doğan & Akaydın. They described a new endemic species, *Acantholimon anatolicum* Doğan & Akaydın, from Ankara province which was at CR category by IUCN due to its very local occurance. The new species was differed from *Acantholimon strigillosum* Bokhari mainly by the differences between habitat preferences, the vegetative organs as habits, leaves, scapes and scales and reproductive organs as spikes, spikelets, bracts, calyx. They stated that *A. anatolicum* grew in deep sandy gypsum, the rich soils at 500 m altitude in Irano – Turanian.

Artelori (1989) studied the 22 populations of three taxa, which are *Limonium ocymifolium* (Poirr.) O. Kuntze, *Limonium graecum* (Poirr.) Rech. and *Limonium virgatum* (Willd.) Fourr. . The area of study was the Kikladhes Islands and the Aegean area of Greece. Artelori studied these taxa, since he claimed that due to comperatively frequent occurance of apomixes in genus *Limonium* and the lack of cytological data of Aegean taxa causes a deficient knowledge about the genus *Limonium* in biosystematic point of view. In order to contribute to reduce this

deficiency about the genus, he studied the cytology and the reproduction of these three taxa and he determined that *L. ocymifolium*, which is endemic to South Greece and occurs in coastal habitats, has a chromosome number as 2n=5x=43 means a pentaploid species; and *L. graecum*, which is widely distributed throughout South Greece, South Aegean region and the coasts of Asia Minor, has a chromosome number 2n=4x=34, means tetraploid, for the two populations out of 5 populations studied of the taxa and the remaining 3 populations of the taxa have a chromosome number 2n=5x=43, means pentaploid. Last species *L. virgatum*, that is apomictic species found in Mediterrenean region and in Western Europe, has chromosome number 2n=3x=27 nd triploid.

In 2007, Zhou and Song studied the blade anatomy structure of four *Limonium* species which were *Limonium gmelinii* (Schrenk) Kuntze, *Limonium otolepis* (Schrenk) Kuntze, *Limonium myrianthum* (Schrenk) Kuntze and *Limonium aureum* (L.) Hill. Blade segregation and paraffin methods were used during the study. As a result of the study, they found out that the blades of these four species had many similar structural adaptations such as having thick cuticle and having anisocytic stomata, mostly being at the same level with the epidermis layer. The glands, which are mostly equilateral and are the characteristics of the family *Plumbaginaceae* also, consists of several cells in upper and lower epidermis and flourishing palisade tissue. In addition, they claimed that the different adaptational features for different plants such as shape differences, stomata frequencies, salt gland frequencies and the thickness of palisade and leaf blade.

In 2008, Doğan, Duman and Akaydın defined a new species of *Limonium*, from Patara Beach, Antalya, Turkey and named it as *Limonium gueneri* Doğan, Duman & Akaydın in section *Limonium*. It grows at sea level in South Western Anatolia like *L. ocymifolium* and its distribution overlaps with *L.ocymifolium*. The two species are both endemic and their phytogeographies are both Mediterrenean. Doğan, Duman and Akaydın claimed that, due to their morphologic, habitat and phytogeographical similarities, *L. gueneri* and *L. ocymifolium* should be closely

related. However, although their distributions are overlapping, while *L. gueneri* is a local endemic that grows on calcareous dry slopes in Eastern Patara Beach; *L. ocymifolium* grows on sandy shores, calcareous and schistose littoral rocks on the shore of Knidos province near Datça; but it has also distributions in mainland and Aegean of Greece as well as Cyprus. In addition, while *L. gueneri* lives at 10-30 m altitude, *L. ocymifolium* lives at 0-5 m altitude.

Plumbaginaceae taxa are generally herbs, climbers, subshrubs, lianes or shrubs with well developed leaves. They are mostly perrenials except the species *L. echioides* (L.) Miller that belongs to the genus *Limonium* Miller. Their leaves are simple, entire to lobed and arranged spirally. They are sometimes auriculate and exstipulate but they are rarely scaly. The leaves of the family *Plumbaginaceae* very often arrange in a rosette and they also show different xeromorphic structures as a grass-like, subulate shape and scleromorphous texture. The genera of *Plumbaginaceae* have differentiations depending on the mesophyll layer of leaves. The mesophyll found in the *Plumbago* L. have cells that are homogenous and rolled while some species of genus *Limonium*, it is not possible to distinct the palisade and spongy parenchyma from each other. In addition, it is possible to see the mesophyll layer at the centre in some species of *Limonium*, *Armeria* and *Acantholimon* Boiss.

Plumbaginaceae taxa are mostly composed of the plants that survive at high concentrations of electrolytes in their environments, which means they are mostly halophytes. Thus one of the most important characteristic of the family is the epidermal glands which secrete mucilage or calcium salts or both of them and these glands may occur on both leaf and stem. Depending on the calcareous matter exluded from some species, these glands may have the name of chalk glands and the calcareous matter that is excluded from these glands can sometimes cover the whole surface of the leaf in some species. These glands may be taken as secretory glands in common and these glands occur in the family are two types. (i) Chalk glands are always found on the leaf lamina or the lamina

surface or just below them. Sometimes simple hairs or warts which are composed of epidermal cells, may surround these glands and the cell walls that lay between those secretory cells and the subsidiary cells are cuticularized. These kind of individual glands are composed of 4 or 8 palisade-like epidermal cells and these chalk glands are also known as Mettenius or Licopoli glands (Metcalfe & Chalk , 1989) (ii) Raised mucilage glands are found in the leaf axils and on the upper surface of the leaf base of the family. Actually the quantitiy of the calcium secreted by the leaf depends on the habitat of the species. Because if a species adapted to a soil type with low calcerous matter, it is not expected for it to secrete calcium from the glands. As an example of this the British species of *Limonium* do not secrete any calcium from the leaves and this property depends on the soil type of the species living on (Metcalfe & Chalk , 1989). Although the function and structure of salt glands are divided into two by Fahn (1988), as the glands that excrete salts by using trichomes in the way of eliminating the salt molecules into vacuole of the bladders cell or as the glands that are located at the epidermal layer and directly excretes the salts to the outside of the cells in which *Limonium* species are located into, also. Although the structure of salt glands varies greately among the different species, it is possible to see similar structure of glands in the same genus or even in the family. This situation is seen mostly in the family of *Plumbaginaceae*. The cellular arrangement of the salt glands and the number of cells that constitute the salt glands are mostly same in most of the *Limonium* species. Fahn (1988) indicated that these glands are composed of complex 16 excretory cells which arranged in four circles, and four large collecting sub-basal cells.

Inflorescences of *Plumbaginaceae* might be racemose, bracteate or thyrsic and they can be also compound or simple. Their bracts are often dry, sheathing and membranous. Flowers are bisexual and often heterostylous. Flowers of the species belonging to family have persistent calyx. The calyx are mostly gamosepalous but they can also be chorisepalous in rare. Petal of the flowers are mostly persistent and they are connate or nearly free. The flowers belonging to species of the family

Plumbaginaceae have often a disc which has sometimes five glands alternating with the stamens. Mostly a persistent calyx encloses the one seeded achene, dry and membranous fruit partially or wholly. The seed includes a straight embryo with mostly an endosperm that include solitary starch grains, however sometimes it is more or less without a starchy endosperm (Kubitzki , 1993). Also there is no perisperm.

In the family *Plumbaginaceae*, the stigma shape has a taxonomic importance first recognised by Boissier, in the classification of the family. By using the shape of the stigma, Boissier seperated *Acantholimon* and *Goniolimon* from the genus *Limonium* (Kubitzki, 1993).

The genera that are included in *Plumbaginaceae* may sometimes include hairs on the leaves which are mostly simple and unicellular but sometimes, particularly, they might be found on the floral organs in the form of long-stalked glandular shaggy-hairs as on the calyx of *Plumbago carpensis* Thunb. and *Plumbago zeylanica* L. (Metcalfe & Chalk , 1989). Stomata developed as ranunculaceous or rubiaceous type (Kubitzki, 1993) and are mostly found on both sides of the leaves but their proportion of distribution on the leaf sides may change with the habitat conditions. For instance stomata of the species *Limonium binervosum* (G.E.Sm.) C.E. Salmon and *Limonium bellidifolium* (Gouan) Dumort. are mostly oriented irregularly but in the species of *Acantholimon* and *Limonium* which have narrow leaves, stomata have pores paralel to the longitudinal axis (Metcalfe & Chalk , 1989).

The family *Plumbaginaceae* is mostly distributed all over the world and their habitat preference are generally cold, arid and saline, coastal environments. The mountaneous, cold and arid environments of the South Asia are the conveniant habitats for the family and these habitat is the centre of genetic diversity of the *Plumbaginaceae* (Kubitzki, 1993). Also in these areas depending on the

isolations due to mountneous habitats, the endemism rates may be expected at higher proportions.

The family *Plumbaginaceae* was first revised by Boissier and informations about the genera and species of the family were studied in different habitats. The family had a place in Flora Orientalis, Flora of Iranica, Flora of Europa, Flora of Italia, Flora of Cyprus, Flora of Russia and Flora of Turkey. The first information of the revision of *Plumbaginaceae* was given in the 4th volume of Flora Orientalis, covering 7 genera and 121 species by Boissier (Boissier, 1879). In Flora Iranica, the family included as 8 genera and 192 species (Rechinger, 1974) , while in 3rd volume of Flora of Europeae *Plumbaginaceae* family is covered 8 genera and 146 species (Tutin and Heywood, 1972). Additionally, in the 2nd volume of Flora of Italia, the family was represented by 6 genera and 58 species (Pignatti, 1982). In the 18 th volume of Flora of Russia, the family *Plumbaginaceae* included 11 genera and 131 species (Komarow, 1967). However, with the studies done in order to describe the family in Flora of Russia, the subgenus of genus *Limonium*, *Myriolepsis* (Boiss.) was defined as independent genus. Furthermore in Flora of Cyprus, the family was represented by 2 genera and 8 species (Meikle, 1985).

In Turkey, *Plumbaginaceae* is represented by 6 genera as *Plumbago* L., *Limonium* Miller, *Goniolimon* Boiss., *Limoniopsis* Lincz., *Acantholimon* Boiss. and *Armeria* Willd and these genera include 51 natural species (Davis et al. 1988). Some of the species from these genera found in the 7th volume of Flora of Turkey are revised and these genera and the number of the species revised are such as; 1 species from *Plumbago* L., 17 species from *Limonium* Miller, 1 species from *Goniolimon* Boiss., 2 species from *Limoniopsis* Lincz, 25 species from *Acantholimon* Boiss and 4 species from *Armeria* Willd. In addition between the years 1982 to 1988 some new other taxa are also described from Turkey (Davis, Mill & Tan, 1988).

Limonium Mill is the largest genus of the family *Plumbaginaceae* throughout the world wide. It has already more than 350 species increasing in number with the incoming new studies but in Turkey *Acantholimon* is the largest genus of the *Plumbaginaceae* (Lledo, Erben & Crespo, 2003).
The two different revisional studies, supported by TUBİTAK, concerning the family *Plumbaginaceae* Juss were lastly done between the years 2001 and 2006. In these research, it was aimed to revise the genera *Acantholimon* Boiss, *Plumbago* L., *Limonium* Miller, *Gonioliomon* Boiss. and *Armeria* Willd. of the family *Plumbaginaceae* Juss. and conducted by Prof. Dr. Musa Doğan and Prof. Dr. Galip Akaydın.

In 2005, Doğan & Akaydın defined another new species belonging to the genus *Acantholimon*, which was named as *Acantholimon evrenii* Doğan & Akaydın. This new species *A. evrenii* grows in East Anatolia, around Elazığ province and the habitat of it seems calcareous mountain slopes at 1200-1600 m height. The flowering time of this species is June. Doğan & Akaydın pointed out that due to its having capitate spike, 2-5 flowering spikelets and heterophyllous leaves, from the perspective of habitat or phytogeography, *Acantholimon evrenii* was close to the species found in the section *Acantholimon*.

The *Limonium* Mill. is also known as the sea lavenders in common and it is the largest genus of the family *Plumbaginaceae* in world wide though the genus *Acantholimon* is the largest genus of the family *Plumbaginaceae* in Turkey. Due to Kubitzki (1993), the estimated number of species of the genus *Limonium* is about 350 but with the studies done on the genus, from 1993 to 2010, it is expected to account more than 350 species described before.

Limonium Mill. includes perrenial dwarf shrubs and herbs except the species *L. echioides* which is the sole annual species of the genus *Limonium*. The flowers of the species of the genus are packed in 3-bractate terminal spikelets (Lledo, Erben & Crespo, 2003) and the calyx is commonly colored although there are species

with the calyx which has no color. The calyx also can be found in various shapes. The petals are often free but there are some species in which the petals are connate at the base. Fruits are mostly utriculate and 1-seeded however rarely pixydate forms are found.

The diversity centre of the species of *Limonium* is mostly in the Mediterrenian and they have important roles in coastal ecosystems (Lledo, Erben & Crespo, 2003). Although the dispersal of the species throughout the world is mostly dominated by the Mediterrenian type climates. However, in Turkey, it is seen that they can disperse through Irano-Turanian type of phytogeographic region also. Actually the distribution of the species of the genus *Limonium* can be seen in a wide range of geographical areas from salt marshes to maritime cliffs and this common distribution of the *Limonium* Mill. is depending on the ability of seed production of the species without any insemination which is known as apomixis. Because of the frequent occurance of apomixis within the genus, the production of the species that are already adapted to the defined environment is greately favored and this situation greately favors the existance of many geographical variants (Lledo, Erben & Crespo, 2003). This also elicits the endemism as well as the high dispersion and adaptation rate with little mophological discontinuity.

Limonium Mill. is represented by 19 species in Turkey (Davis, 1982). Ten of the taxa that are found in Turkey grows only at the saline lands next to marine environments. This accounts of the 37% of the all taxa in Turkey are growing only in the salty environments of marinelands. The species that are found at these saline environments of marinelands are *L. sinuatum* (L.) Miller, *L. angustifolium* (Tausch) Turrill, *L. ocymifolium* (Poirr.) O. Kuntze, *L. virgatum* (Willd.) Fourr, *L. graecum* var. *graecum* (Poirr.) Rech., var. *hyssopifolium* (Girard) Bokhari, *L. sieberi* (Boiss.) O. Kuntze, *L. didimense* Akaydın & Doğan, *L. marmarisens*e Akaydın& Doğan and *L. guenerii* Doğan, Duman & Akaydın (Doğan and Akaydın, 2006). And the species *L. vanense* (Kit Tan & Sorger), *L. meyeri* (Boiss.) O. Kuntze, *L. caspium* (Willd.) Gams, *L. iconicum* (Boiss. & Heldr) O.

Kuntze, *L. tamaricoides* Bokhari, *L. lilacinum* var. *lilacinum* (Boiss. & Bal.) Wagenitz, var. *laxiflorum* Doğan & Akaydın, *L. pycnanthum (C. Koch) O. Kuntze*, *L. globuliferum* var. *globuliferum* (Boiss. & Heldr.) O. Kuntze, var. *subglobosum* Doğan & Akaydın, *L. anatolicum* Hedge, *L. smithii* Doğan & Akaydın and *L. davisii* Doğan & Akaydın are making of the thirteen taxa that are occupying the saline inlands of Anatolia and this accounts for the 48% of the all taxa belonging to *Limonium* Mill. that are found in Turkey, which are growing in the saline inland areas. The remain of the taxa, that are found in Turkey, are *L. gmelinii* and *Limonium effusum* (Boiss.) O. Kuntze. These taxa can both grow and live in the saline or arid environments that can be found in terrestrial and marine habitats (Doğan & Akaydın, 2006).

In addition, 14 of the taxa, which makes up the 52% of the total taxa found in Turkey, are endemic to Turkey. The endemic taxa of the genus *Limonium* Mill, found in Turkey are as follows: *L. vanense, L. didimense, L. marmarisense, L. guenerii, L. effusum, L. iconicum, L. tamaricoides, L. lilacinum* var. *lilacinum,* var. *laxiflorum, L. pycnanthum, L. anatolicum, Limonium smithii* and *Limonium davisii.*

In the 7th volume of Flora Of Turkey (Davis,1982), Bokhari and Edmonson divided the genus *Limonium* into 5 sections as *Pteroclados*, *Limonium*, *Sphaerostachys*, *Sarcophyllum* and *Schizyhymenium*. Due to this grouping, the section *Pteroclados* only includes *Limonium sinuatum*,the section *Sarcophyllum* includes only *L. anatolicum* and the section *Schizyhymenium* only includes *L. echioides* while section *Limonium* includes *L. gmelinii*, *L. angustifolium*, *L. meyeri*, *L. effusum*, *L. ocymifolium*, *L. virgatum*, *L. graecum*, *L. sieberi*, *L. bellidifolium*, *L. iconicum* and *L. tamaricoides*. In addition, the section *Sphaerostachys* includes *L. lilacinum*, *L. pycnanthum* and *L. globuliferum*.

Throughout the taxa of genus *Limonium* Mill. found in Turkey, 55% of the all taxa are categorized in CR by IUCN which corresponds to 15 of the all taxa

found in Turkey. 33.3% of the total taxa which corresponds to 9 of the whole taxa found in Turkey, are in the EN category. Because *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* have populations with narrowly dispersed and low members, they are put into the CR category by IUCN. In addition *L. sinuatum*, *L. angustifolium*, *L. effusum*, *L. virgatum*, *L. sieberi*, *L. bellidifolium*, *L. iconicum*, *L. globuliferum* var. *globuliferum* and var. s*ubglobasum* have populations which have a probability to be in danger in the future, thus they are in the EN category by IUCN. For further information, since *L. meyeri* and *L. echiodies* have populations with low members and are found only at specific locations in Turkey, they are categorized in VU by IUCN. However, since *L. gmelinii* are commonly dispersed in the saline terrestrial and saline marine environments in Turkey, it is categorized in LC by IUCN.

In this study, it is aimed to examine leaf anatomy of *L. echioides*, *L. globuliferum*, *L. tamaricoides*, *L. anatolicum* and *L. sinuatum*, which are belonging to five different sections due to Davis (1982). By examining these five taxa belonging to five different sections, it is aimed to find out if there is another differentiating properties of these five sections within each other. Also it is intended to differentiate between the Mediterrenean type taxa as *L. echioides* and *L. sinuatum* and Irano-Turanian type taxa as *L. globuliferum*, *L. tamaricoides* and *L. anatolicum.* In addition, it is aimed to elucidate the differences and significant properties of halophytes.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

The plant materials of *L. globuliferum, L. echioides*, *L. tamaricoides*, *L. anatolicum* and *L. sinuatum,* used in this study were collected from Turkey (Davis and Heywood ,1973) between 2002-2005. Specimens were obtained from the herbarium of Department of Biological Sciences, Middle East Technical Univercity, Ankara. The collector numbers and localities of the specimens are given in Table 2.1.

Table 2.1 Leaf type, phytogeography, specimen numbers and the collection area of the given taxa.

Taxon	Leaf Type	Phyto geography	Specimen Numbers	Collection Area
Limonium echioides	Basal	Mediterrenean	G.Akaydın 10269	C4- Mersin, Silifke, İncekum Foreland, Göksu Delta, Around bird observation tower Sandy beaches – 3mt
Limonium globuliferum	Basal	Irano-Turanian	G.Akaydın 9227	B5 – Beneath the Seydişehir Village Sandy prairie – 1070 mt
Limonium tamaricoides	Basal	Irano-Turanian	G.Akaydın 9237	Around the Seyfe Lake, Sandy areas
Limonium anatolicum	Shoot	Irano-Turanian	G.Akaydın 7684	B4- Ankara,About 8 km at Ankara road, Around Salt Lake Area Salty (stepe) Marshes – 940 mt
Limonium sinuatum	Basal Shoot	Mediterrenean	G.Akaydın 7846	B1- İzmir Karaburun, at Küçükbahçe Road about 10 km, maquis around the road – 15 mt

Throughout these species, while *L. echioides*, *L. globuliferum* and *L. tamaricoides* have basal leaves, *L. anatolicum* has only shoot leaves and *L. sinuatum* has both shoot and basal leaves.

2.2. Methods

In this study, the paraffin sectioning method (Johanson; 1944) was used in order to observe the diagonistic anatomic leaf characteristics of the species.

Since the plant materials used in the thesis were dry samples, before starting the experiments, they were put into distilled water and then into incubator (Nuve Incubator EN 055) and stayed at 60 ºC for one or two days, in order to soften the tissues for the experiments. After this softening procedure, the tissues were dissected to be minimized for being in suitable sizes for the cassettes (microtome blocks).

After dissection; dehydration, clearing, infiltration, embedding, sectioning, hydration, staining, dehydration and the permanizing procedures were done in sequence (Metcalfe & Chalk, 1989).

In this study, surface of the leaf specimens were also taken in order to define the stoma types of the different species as well as to find out the average stomata number per unit leaf and average stomata length and width.

The dry specimens that were put into incubator at 60 ºC for being softened, were taken out and put into %70 alcohol in order to prevent them from shrinkage.

2.2.1. Surface View

The dry specimens that were put into incubator at 60 ºC for being softened were taken out and put into %70 alcohol in order to prevent them from shrinkage.

For each species, at least 4 leaves were used. Then, both the upper and lower surfaces of these leaves were gathered with a sharp blade. The slides, prepared with a drop of distilled water were investigated under light microscope (Leica DM 1000).

2.2.2. Staining

In this procedure safranin was used as a dye. A drop of safranin was put over the edges where the cover slip was combined with the slide and waited until the tissue took the stain.

In this study, staining step was eliminated since the stomata were easily observed under the microscope without safranin.

2.2.3. Photographing

The observations were obtained by using a Leica DFC 280 model of camera which was attached to a Leica DM 1000 model of light microscope. In order to find out the avarage stomata number per unit area of a leaf and average stomatal length and width; the stomata number, lenght and width were measured under microscope at 40 X magnification.

2.2.4. Paraffin Sectioning Method

2.2.4.1 Dehydration

In order to make good sectioning, the tissues must be embedded into paraffin. However, the paraffin is not miscible with water. Thus, the extractable water in the tissues was removed by doing dehydration. To achieve this procedure, the tissues were put into increasing strength of alcohol solutions as 50%, 70%, 80%, 100% for about 30 minutes.

The reason of using increased level alcohol was to prevent the distortion of the tissue due to shrinkage.

2.2.4.2. Clearing

Clearing procedure was employed so as to remove the dehydrant. Since the paraffin also was not miscible with alcohol, the extractable alcohol must be removed from the tissue and it must be switched with another solute such as xylene, which was mixible with the paraffin. Here the important point was, the antimedia used during this clearing procedure must be miscible also with the media of dehydration , here EtOH.

With this procedure, the tissue were cleared and the antimedia, xylene, was loaded through them. In order to load xylene through the tissues, the xylene-alcohol solutions were used with increased level of xylene concentrations. The samples were put in to 2:1 alcohol-xylene, 1:1 alcohol-xylene, 1:2 alcohol-xylene and pure xylene solutions for 30 minutes respectively.

2.2.4.3. Infiltration

This step was done in order to penetrate the paraffin into the tissues while xylene was removed. After putting the tissues into pure xylene and waited for about 30 minutes, immediately paraffin was added over the samples and these samples were left in room temperature for one night. After leaving the tissues at room temperature for one night, the tissues were taken and put to incubator at 60ºC and waited until the smell of xylene was totally removed from the tissues. The time period of this step of the experiment changed due to the properties of the samples that were used or the amount of the xylene left on the sample before the paraffin was added over.

2.2.4.4. Embedding

The samples, from which the xylene was totally removed, were ready to be embedded into the base molds. During this procedure, it was important to embade the selected tissue perpendicular to the base of the base mold and during embedding it was important to be quick, since if the paraffin got cooler, there was going to be a layer of paraffin which might gone into pieces during sectioning. After covering the whole tissue with parafin, the base molds were put over a smooth place to cool; and after, they were put into refregirator untill they were going to be sectioned.

In this step, placing the tissues into the paraffin in perpendicular to x axis was very important since if the tissue stayed curved, during sectioning procedure, the samples could not be taken properly.

2.2.4.5. Sectioning

Before paraffin sectioning, a solution that sticks the sample to the slide was prepared. During this study, in order to adhere the microtome samples to the slides, egg albumin was used. It was highly important to get rid of all the bubbles while applying the egg albumin onto the slides; because if there remained any bubles, there would be a probability of overlapping them with the microtome samples and this might cause the cross-sections of the samples being disrupted. After preparing the adhesive solution of egg albumin, the water bath was prepared at 50 ºC.

In this study, 10 - 15 µ thickness of leaf sections were taken with the help of Leica RM2125RT model of Rotary Microtom. The range of the thickness changed depending on the hardness of the material.

After taking the sections by using the microtome, they were put onto the water bath (Apex) at 50ºC. The slides, covered sole face with egg albumin, were used to take the sections from the water bath and put to dry.

2.2.4.6. Hydration

For staining these dehydrated tissues, water must be added to them. However, here the tissues also have paraffin. Because of this reason, the sections on the slides were put into incubator at 60 ºC over night. Then immediately, they were put into the absolute xylene to remove all the paraffin from the tissues. The xylene started to resolve the paraffin and led the sole section of the tissue remained on the slides. After xylene, the tissue were put into 2:1 xylene:alcohol, 1:1 xylene:alcohol, 1:2 xylene:alcohol, 100% alcohol, 80% alcohol, 70% alcohol, 50% alcohol solutions in turn for about 5 minutes for each step.

2.2.4.7. Staining

Staining step was done in order to create a contrast between the tissue parts of the sections and to observe the tissue easily under the microscope. In this study, safranin solution was used to stain the leaf sections.

After taking the slides from 50% alcohol solution, they were put into safranin solution for about 1 minute. Then, they were washed under running tap water for about a minute and put into 50% alcohol solution to remove the excess dye.

2.2.4.8. Dehydration

To make permanent slides, water taken by hydration step was removed with the help of dehydration process. So, the slides (the leaf sections) were prevented to be damaged and distorted from the shrinkage

The slides were put into 50%, 70%, 80%, 100% of alcohol solutions and 2:1 alcohol:xylene, 1:1 alcohol xylene, 1:2 alcohol:xylene and absolute xylene solutions respectively one after another immediately.

In this procedure, the dehydrated slides may remain in pure xylene until they are going to be permanized.

2.2.4.9. Permanizing

Permanization was done in order to protect the samples from environmental damages such as microbial attacks, humidity etc.

To make permanized slides, Entellan was used as a mounting solution. It was important to be quick in this step, because due to high votality of xylene, the slides were easily drying after taking them out from xylene solution. However, during mounting, the slides must not to be dry.

After putting a drop of entellan onto the slide, immediately the cover slip was put over the slide and entellan spreaded through all the sample. Then, the slides were controlled if there remained any bubble inside.

Bubbles were important air cavities that might halt all the procedure, since they might force entellan to get out from the slide during drying and might disrupt the permanization procedure, since to observe the samples with no entellan over them, were not possible to be seen.

It could be especially noted that, if there remained any air bubble over the sample, it was not possible to observe the sample under the microscope because these samples were going to be seen as black dots only. Thus by using the back of forceps, bubbles were forced to exit from the slide. After this procedure , the slides were put to room temperature for drying.

It should be notted that, before putting the slides into slide boxes to store or before observing them under the microscope; they were frequently controlled to see that if they were totally dried and permanized.

2.2.4.10. Photographing

The mid veins and side veins of the leaf specimen were looked under Leica DM 1000 light microscope and the photos were taken by the Leica DFC 280 camera at 40 X magnification and then labelled. The entire leaf specimen photos were taken by Leica DFC 280 camera at 4X and 10X magnifications depending on the size of the specimens.

2.2.5. Calculations

The stomatal length and stomatal width for each specimen were measured and written over a coloumn in Microsoft Office Excell 2007. The number of stomata per 210.68µm x 263.27µm leaf area for each specimens were counted and written over a coloumn in Microsoft Office Excell 2007. Then the average and the standard deviations of the results were calculated.

CHAPTER 3

RESULTS

3.1. *Limonium echiodes*

Figure 3.1. *L. echioides* by Galip Akaydın.

3.1.1. Lower Epidermis

The type of the stomata depending on the epidermal cells are anisocytic and the average number of stomata found per 210.68µm x 263.27µm surface of leaf is 4.05 ± 1.36. The average length of stomata is 30.70 ± 3.94 µm. The average width of stomata is 26.46 ± 2.78µm.

Table 3.1 Length of the stomata of lower epidermis of *L.echioides* leaf in µm

25.54	29.18	36.37	23.36	35.48	38.24	27.61	32.79	29.51
24.6	34.47	31.98	23.53	32.85	27.95	25.44	35.26	28.62
23.98	36.37	32.23	24.29	36.37	34.99	24.28	33.9	35.49
23.83	31.57	33.54	28.37	31.98	29.32	28.13	36.54	27.07
25.57	32.23	31.5	25.27	32.23	27.45	25.48	33.57	28.94
35.5	36.37	28.06	27.73	33.54	34.62	29.18	35.5	27.72
33.57	32.23	28.48	27.14	31.5	30.63	31	34.51	27.59
36.54	35.48	34.47	24.89	38.24	24.93	32.79	34.47	26.23
34.51	31.57	29.08	25.57	29.18	34.03	35.26	33.13	29.88
33.13	32.85	26.14	29.16	31	28.74	33.9	29.18	33.3
29.43	29.1	27.04						
Avarage = 30.7027956989247								
Std. Dev. = 3.94132822612748								

Table 3.2 Width of the stomata of lower epidermis of *L. echioides* leaf in µm

26.58	28.9	28.59	23.13	30.4	24.49	24.83	23.88	26.99	30.54
26.64	28.89	27.36	24.55	28.27	25.02	26.22	26.58	28.15	27.7
26.71	30.13	27.76	26.42	23.25	23.35	24.63	24.85	27.62	25.86
24.56	28.58	25.94	25.38	23.98	25.93	26.12	25.42	23.41	29.95
27.34	25.13	26.85	25.65	25.06	24.6	23.94	26.57	26.98	20.42
27.64	31.57	26.58	21.69	22.37	24.18	26.71	25.46	26.67	31.02
28.17	30.13	23.76	28.14	25.47	29.47	22.48	27.33	23.5	39.32
24.72	28.62								
Avarage = 26.4597222									
Std. Dev. = 2.78301992837862									

Table 3.3 Number of stomata per 210.68µm x 263.27µm area of *L. echioides* leaf

4	4	6	4	3	4	4	6	4	3
3	5	4	4	7	6	2	2	2	3
5	4								

Avarage = 4.04545454545455

Std. Dev. = 1.36197590102702

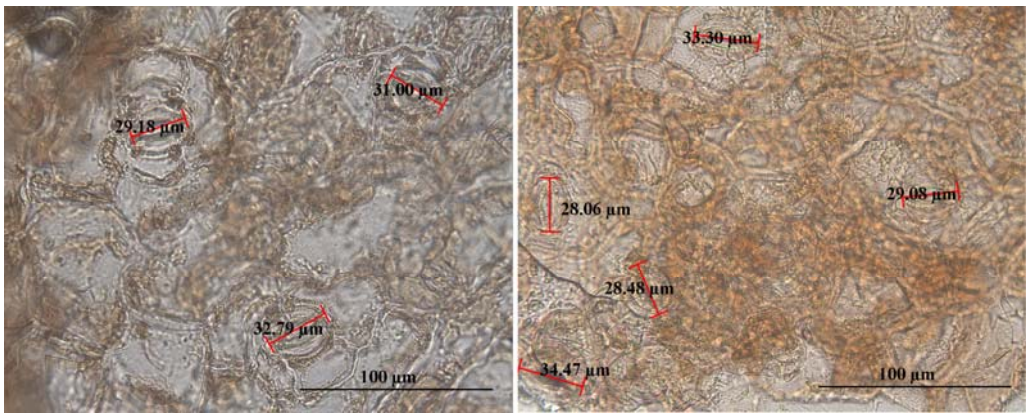

Figure 3.1.1. Photo of two samples of stomatal length of *L. echioides* (40X) (lower epidermis).

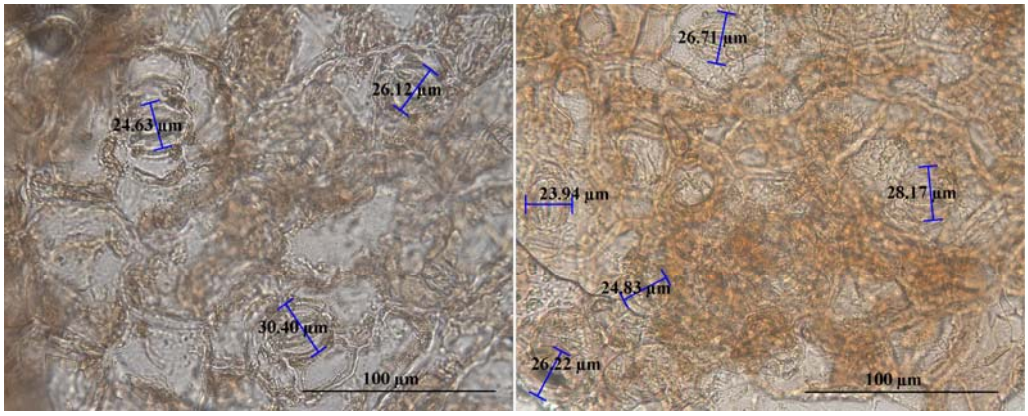

Figure 3.1.2. Photo of two samples of stomatal width of *L. echioides* (40X) (lower epidermis).

3.1.2. Upper Epidermis

The type of the stomata depending on the epidermal cells is anisocytic. The average number of stomata found per 210.68µm x 263.27µm surface of leaf is 3.86 ± 1.17. The average length of stomata is measured as 29.93 ± 3.72 µm. The average width of stomata is 25.61 ± 3.77 µm.

Table 3.4 Length of the stomata of upper epidermis of *L. echioides* leaf in µm

31.48	29.54	32.36	29.6	22.58	28.5	36.74	23.72	33.01
28.89	28.32	32.55	33.57	30.25	25.43	18.31	28.19	33.35
28.51	24.72	30.23	28.89	32.03	29.37	35.64	28.67	33.69
31.29	29.11	33.41	31.92	25.94	25.19	32.98	25.19	32.38
32.51	30.21	35.41	30.73	25.97	33.72	28.16	30.13	32.54
27.85	30.72	36.58	32.56	24.77	30.27	29.37	33.84	29.54
31.85	26.68	30.15	31.83	23.16	28.94	29.22	28.52	32.21
30.39	32.1	35.37	32.86	27.08	28.89	29.08	28.54	33.42
36.96	20.6	32.26	29.1	21.4	34.87	30.01	31.95	33.34
25.92	32.51	24.85						

Avarage = 29.9344047619048

Std. Dev. = 3.72336560770643

Table 3.5 Width of the stomata of upper epidermis of *L. echioides* leaf in µm

24.16	23.53	25.77	19.72	25.13	21.14	26.03	17.98	27.78	27.36
25.25	25.99	23.41	23.94	28.43	21.79	28.01	15.48	24.61	26.43
26.64	28.25	25.35	21.87	26.67	29.89	25.64	20.95	21.18	23.09
29.56	24.68	25.3	17.72	27.67	34.88	28.62	20.08	27.39	28.91
29.01	24.54	19.85	23.49	22.76	27.39	27.83	29.74	25.26	
25.79	26.99	25.85	24.46	27.78	27.35	23.01	25.7	26.52	
28.01	20.35	34.62	25.07	27.9	26.3	26.91	23.63	26.06	
32.79	28.48	14.62	27.08	28.49	31.24	26.03	23.46	26.13	
32.29	30.23	29.06	20.39	25.4	26.85	29.8	19.73	26.04	

Avarage = 25.6065882352941

Std. Dev. = 3.77269783252958

Table 3.6 Number of stomata per 210.68µm x 263.27µm area of *L. echioides* leaf

3	5	3	3	3	5	3	7	3	5
2	2	4	3	5	4	4	4	5	4
4	4								

Avarage = 3.86363636363636

Std. Dev. = 1.16682126372117

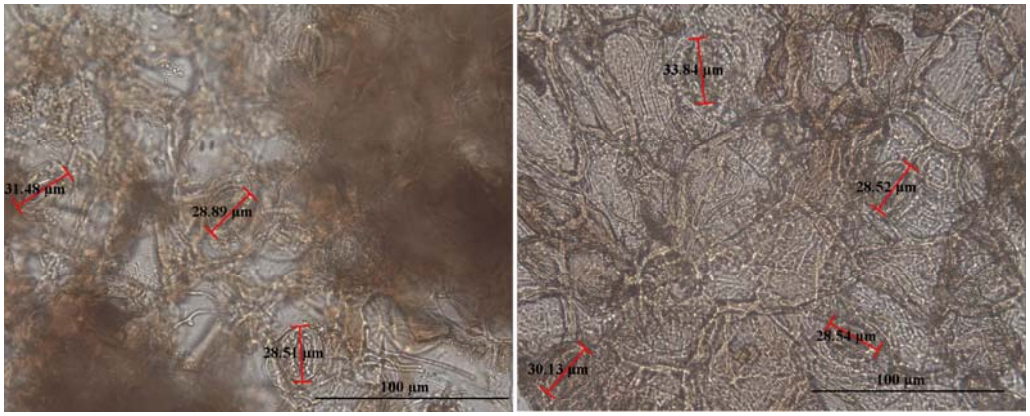

Figure 3.1.3. Photo of two samples of stomatal length of *L. echioides* (40X) (upper epidermis).

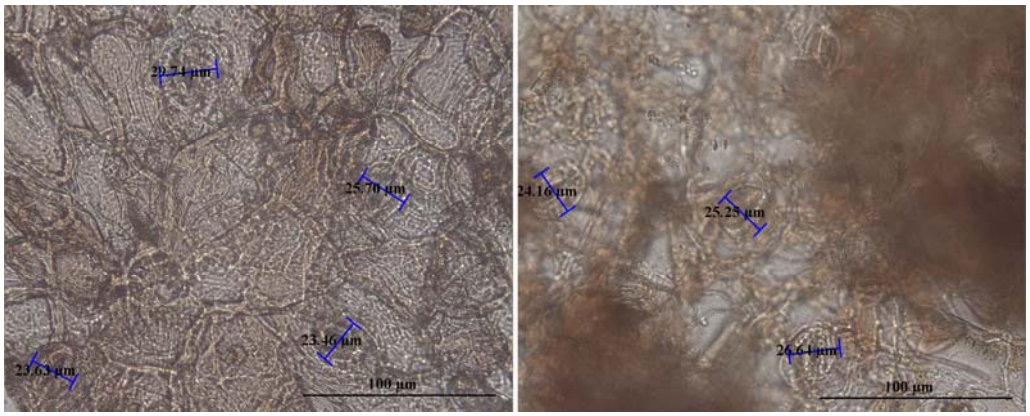

Figure 3.1.4. Photo of two samples of stomatal width of *L. echioides* (40X) (upper epidermis).

The stomatal length and stomatal width of the upper and lower epidermis are found to be approximately same. The stomatal density per 210.68µm x

263.27µm are of leaf surface is also approximately equal to each other for upper and lower epidermis.

3.1.3. Cross Sectioning

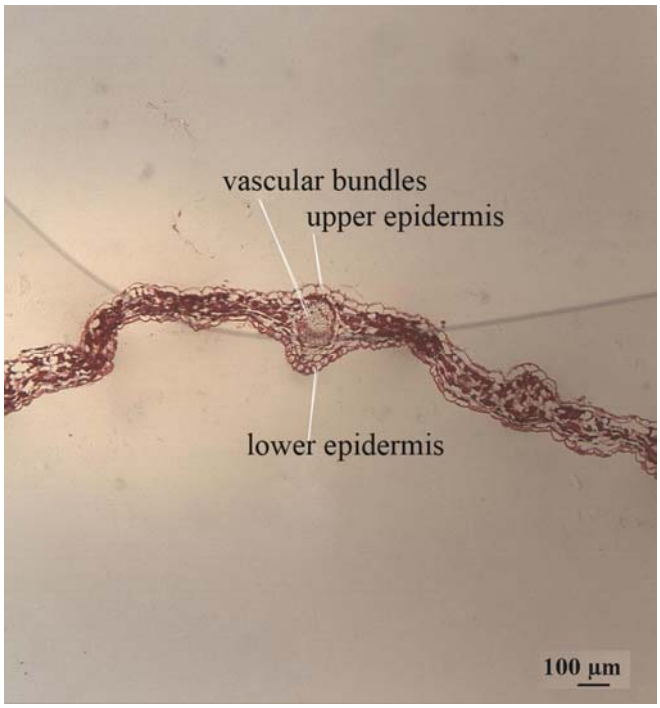

Figure 3.1.5. Leaf of *L. echioides* (4X) in cross section

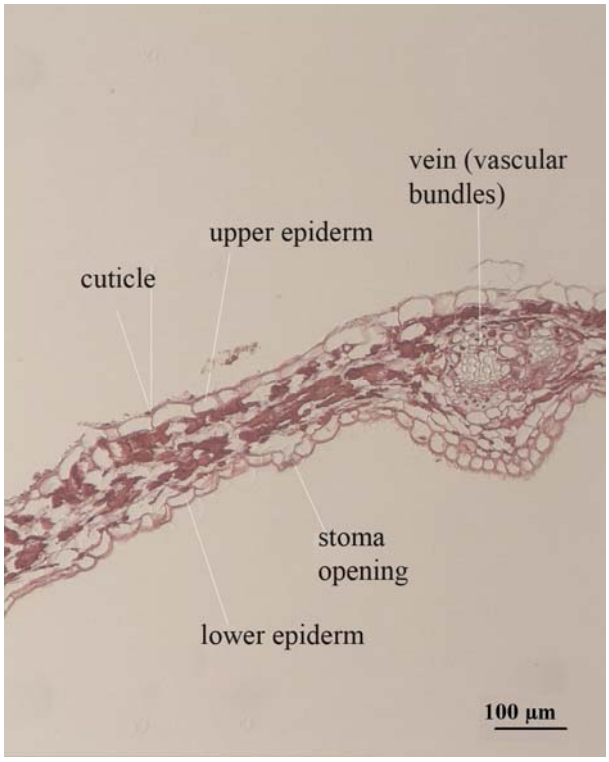

Figure 3.1.6. Cuticle, upper epiderm, lower epiderm and stoma openning on the leaf of *L. echioides* (10X) in cross section

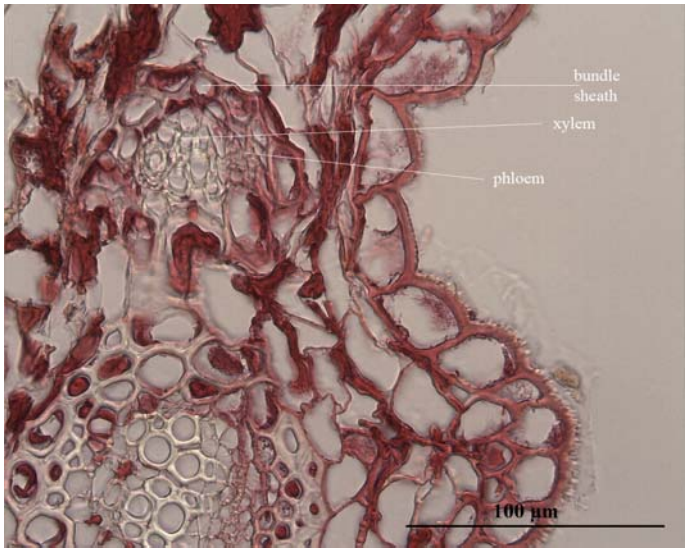

Figure 3.1.7. Bundle sheath cells, phloem and xylem (vascular bundles) of subsidiary vein belonging to leaf of *L. echioides* (40X) in cross section

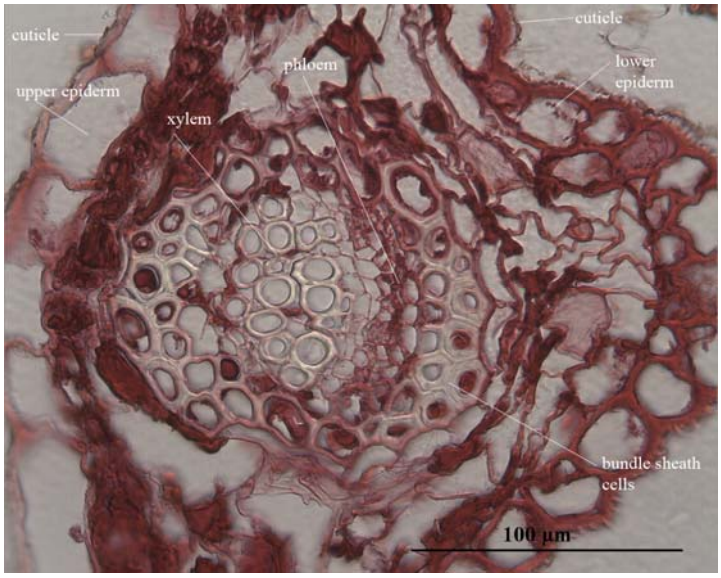

Figure 3.1.8. Phloem, xylem, cuticle, upper and lower epiderm and bundle sheath cells of midrib of leaf of *L. echioides* (40 X) in cross section

L. echioides leaf has one layered upper and lower epidermis. The xylem and phloem is covered by bundle sheath parenchyma cells and the phloem of vascular bundles is nearer to lower epidermis than the xylem. The significant cuticle layer is seen both over the lower and upper epidermis. Subsidiary vein of the leaf is only explicit for one side of the leaf while the other subsidiary vein is not clearly seen in sectioning specimen.

3.2. *Limonium globuliferum*

Figure 3.2. *L. globuliferum* by Galip Akaydın.

3.2.1. Lower Epidermis

The type of the stomata depending on the epidermal cells are anisocytic. The average number of stomata found per 210.68µm x 263.27µm of leaf surface is 3.54 ± 1.14. The average length of stomata is 32.28 ± 3.84 µm. The average width of stomata is 22.88 ± 3.34 µm.

Table 3.7 Length of the stomata of lower epidermis of *L. globuliferum* leaf in µm

22.22	31.49	33.54	29.07	35.98	34.3	36.9	31.57	34.88
24.01	33.6	37.38	31.08	31.07	30.79	35.12	31.14	32.86
27.32	26.75	28.81	34.91	35.48	34.19	35.6	27.29	36.53
25.82	31.56	31.86	33.68	34.45	35.04	37.83	30.97	34.54
26.42	27.8	33.7	29.04	37.65	31.14	39.6	32.23	30.59
33.07	29.78	22.89	34.2	35.94	34.54	30.72	33.51	32.91
32.04	33.57	29.93	30.58	33.67	35.83	25.23	31.27	28.92
30.66	26.27	34.52	27.33	31.79	37.46	31.15	35.44	33.1
36.2	29.31	34.06	29.05	34.04	35.9	36.48	29.27	32.98
25.09	32.97	29.84	35.98	32.97	35.6	31.64	31.07	27.55
29.36	29.76	34.62	31.28	32.54	34.19	25.34	32.83	35.83
27.47	29.19	31.92	33.8	38.09	35.07	22.76	33.56	37.72
35.19	28.74	37.13	35.77	38.26	35.94	28.78	32.94	36.2
35.38	27.95	40.99	36.88	36.52	21.58	35.81	33.03	32.36
30.13								

Avarage = 32.2758267716535
Std. Dev. = 3.84137013439689

Table 3.8 Width of the stomata of lower epidermis of *L. globuliferum* leaf in µm

15.57	20.3	24.87	21	24.26	25.11	22.78	24.29	29.16
21.98	21.73	25.79	21.71	21.88	22.27	22.54	23.2	19.35
24.76	23.35	23.08	19.5	24.37	25.39	26.96	19.11	23.47
21.47	22.01	25.31	22.07	18.9	21.5	21.32	20.39	23.38
26.85	19.29	27.44	21.97	22.33	20.89	22.35	21.71	22.99
20.85	20.96	26.3	22.05	25.13	19.18	24.19	18.05	23.24
22.24	21.42	28.17	20.88	24.99	19.1	21.28	23.57	23.98
20.85	22.13	25.88	21.69	24.94	20.72	25.37	22.81	20.35
22.21	24.23	24.97	21.47	22.16	21.66	20.92	23.48	22.04
23.5	21.58	25.2	23.98	23.54	23.19	17.62	18.19	21.09
19.56	18.53	19.56	26.3	24.556	20.02	22.99	21.4	40.44
26.33	27.32	19.93	25.38	19.46	21.24	26.05	17.49	31.83
31.57	24.83	20.71	26.39	22.72	24.45	17.08	22.22	32.91
24.07	26.25	20.04	21.09	18.21	21.68			

Avarage = 22.8772845528455
Std. Dev. = 3.33882300975921

Table 3.9 Number of stomata per 210.68µm x 263.27µm area of *L. globuliferum* leaf

4	5	2	4	2	3	4	2	2
4	5	4	4	3	3	3	3	2
4	4	5	4	4	4	3	2	2
5	4	3	3	3	5	6	2	4
4	6	2						

Avarage = 3.53846153846154

Std. Dev. = 1.14354374979373

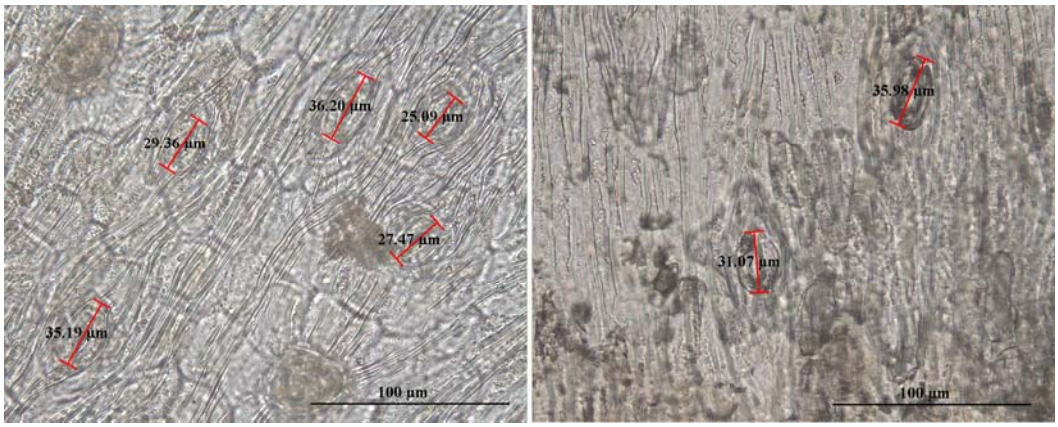

Figure 3.2.1. Photo of two samples of stomatal length of *L. globuliferum* (40X) (lower epidermis).

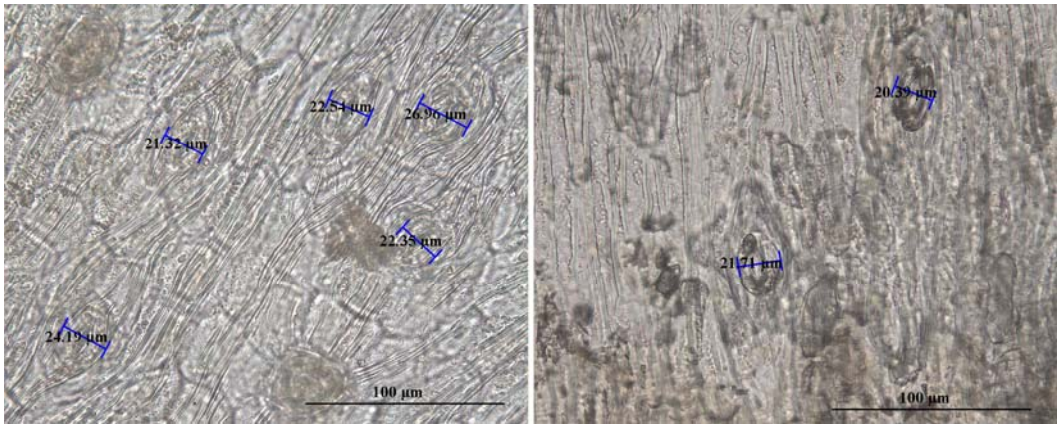

Figure 3.2.2. Photo of two samples of stomatal width of *L. globuliferum* (40X) (lower epidermis).

3.2.2. Upper Epidermis

The type of the stomata depending on the epidermal cells are anisocytic. The average number of stomata found per 210.68µm x 263.27µm leaf surface is 6.91 ± 1.95. The average length of stomata is 31.18 ± 3.91 µm. The average width of stomata is 24.95 ± 3.08 µm.

Table 3.10 Length of the stomata of upper epidermis of *L. globuliferum* leaf in µm

28.35	31.83	32.56	25.13	29.99	35.18	25.78	38.12	33.59
31.84	33.66	27.9	26.08	29.35	36.42	29.66	36.89	32.95
29.4	35.84	34.98	26.98	24.98	31.65	26.3	34.27	36.31
25.19	35.9	35.98	29.07	33.78	38.17	27.41	35.52	35.17
31.71	36.83	35.25	30.35	27.4	33.98	27.23	32.94	32.21
29.42	38.18	34.76	33.1	26.03	34.23	29.78	30.46	31.06
30.75	35.75	37.35	31.23	29.78	36.03	28.86	34.49	33.13
27.59	36.28	35.93	30.32	27.15	31.73	26.56	29.53	35.18
27.63	33.44	33.5	31.2	19.82	35.43	24.76	35.7	33.82
29.23	33.43	33.52	28.6	31.78	33.91	26.46	30.27	34.73
31.45	32.7	37.43	29.82	32.75	32.27	28.02	27.4	33.8
21.47	36.73	36.66	25.9	31.78	34.23	25.31	28.61	39.05
25.65	27.72	32.31	29.43	30.74	31.24	26.9	33.41	39.44
25.76	32.23	33.88	28.79	33.95	24.98	24.95	31.73	34.8
25.39	29.28	28.01	27.2	28.86	29.62	28.31	30	28.25
28.25	28.15	28.34	28.59					

Avarage = 31.1763309352518

Std. Dev. = 3.9066782553901

Table 3.11 Width of the stomata of upper epidermis of *L. globuliferum* leaf in µm

23.39	26.07	27.6	25.22	23.14	24.89	24.6	27.23	20.66
21	27.61	24.92	24.89	21.75	30.55	24.95	29.81	28.9
24.23	28.01	22.53	26.05	19.97	25.55	23.47	28.77	23.32
27.45	26.22	25.29	26.67	25.31	30.51	26.16	20.89	23.41
26.95	23.56	22.55	27.2	18.56	27.08	22.47	22.33	22.01
22.7	26.73	28.71	25.65	28.33	24.6	21.86	31.68	22.34
20.35	26.13	25.58	28.52	27.24	27.73	23.95	22.53	20.47
28.69	25.54	23.8	27.6	21.24	28.38	29.48	25.25	25.94
22.87	27.28	19.19	26.79	25.07	22.4	26.75	20.7	30.78
21.87	27.34	22.58	27.93	30.18	27.46	20.96	22.21	26.59
25.38	22.71	22.57	26.63	22.39	25.29	21.21	20.89	20.82
19.67	30.39	23.54	27.29	21.43	25.5	25.88	21.41	23.56
26.71	30.15	29.63	26.77	24.68	26.51	26.24	22.61	21.83
20.61	25.92	22.09	27.73	18.82	27.07	24.51	27.04	27.85
23.93	27.15	21.17	27.56	20.89	25.01	30.53	23.18	19.72
19.88	20.68	25.49	25.95	23.94	36.02	24.01	20.89	25.9
24.56	26.48	22.45	26.68	24.33	30.02	25.46	23.7	25.38

Avarage = 24.9539869281046
Std. Dev. = 3.08461512140431

Table 3.12 Number of stomata per 210.68µm x 263.27µm area of *L. globuliferum* leaf

9	8	8	5	9	10	6	7	3
7	9	5	6	9	9	5	8	7
7	6	3	5	8				

Avarage = 6.91304347826087
Std. Dev. = 1.95199300199614

Although the stomatal length and the stomatal width of the upper and lower epidermis of the leaf of *L. globuliferum* is almost equal, there is a 4 units difference between the stomatal density of upper and lower epidermis. The upper epidermis has more stomatal density when compared with the lower epidermis.

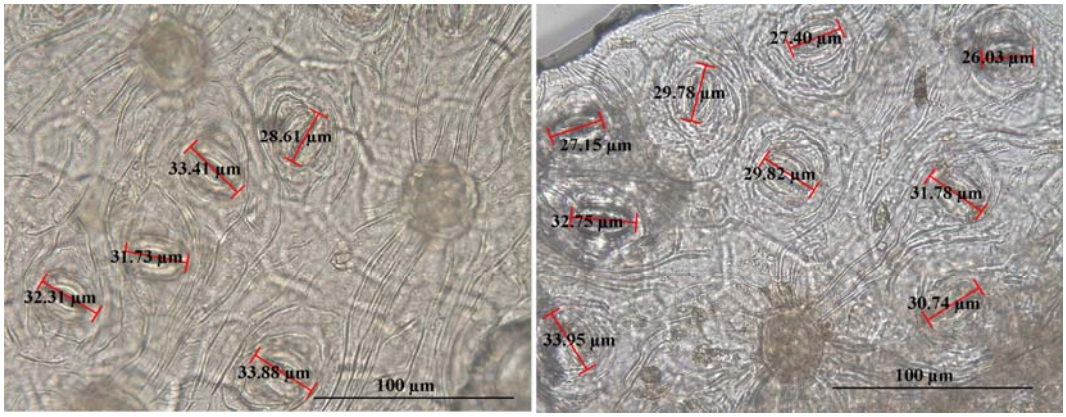

Figure 3.2.3. Photo of two samples of stomatal length of *L. globuliferum* (40X) (upper epidermis).

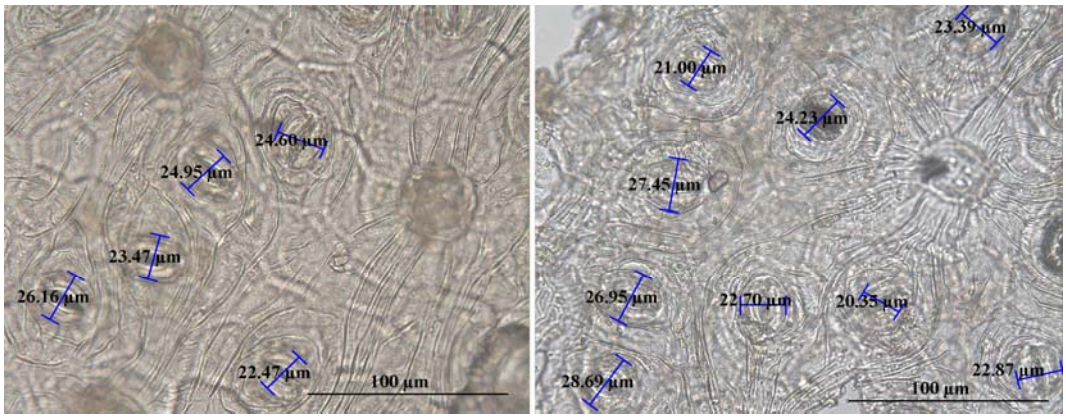

Figure 3.2.4. Photo of two samples of stomatal width of *L. globuliferum* (40X) (upper epidermis).

3.2.3. Cross Sectioning

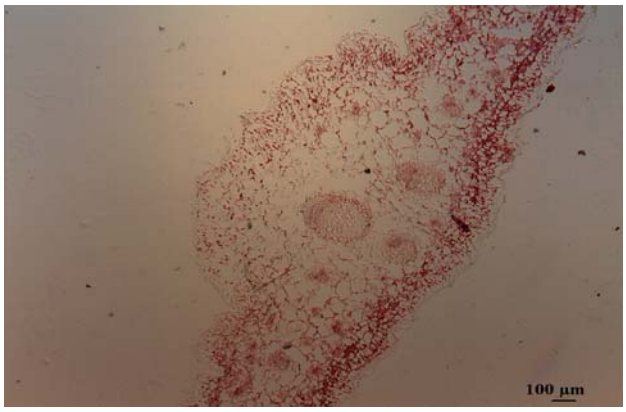

Figure 3.2.5. One midvein and three subsidiary veins in midrib of leaf of *L. globuliferum* (4X) in cross section.

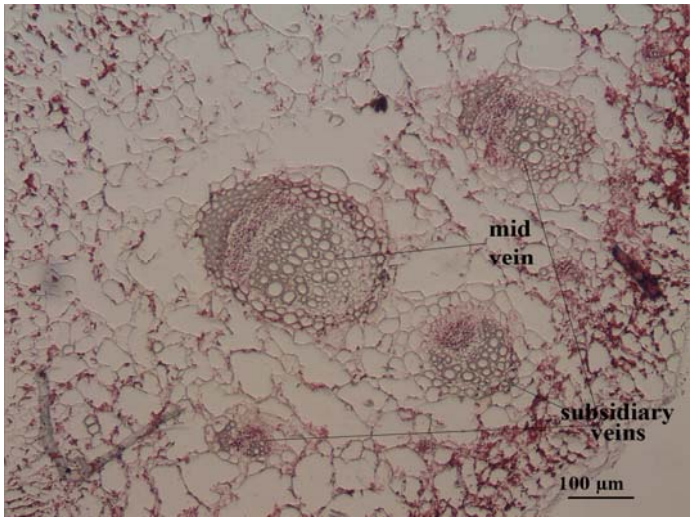

Figure 3.2.6. Midvein and three subsidiary veins in leaf of *L. globuliferum* (10 X) in cross section.

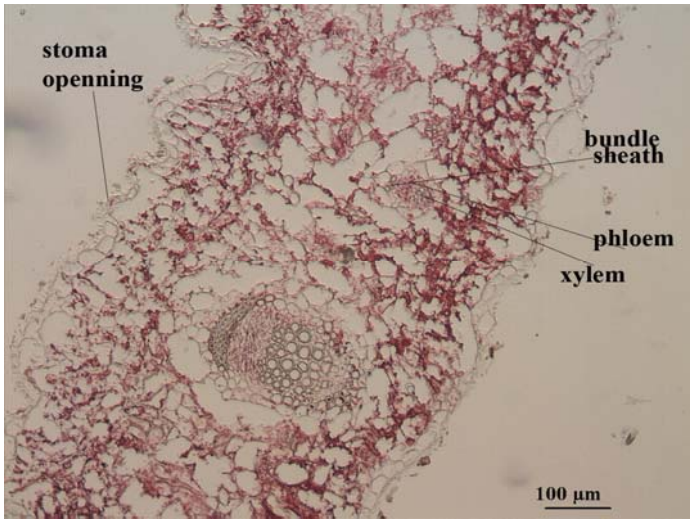

Figure 3.2.7. Xylem, Phloem and Bundle Sheat cells of the lateral rib next to vein, placed next to midrib of leaf of *L. globuliferum* (10X) in cross section.

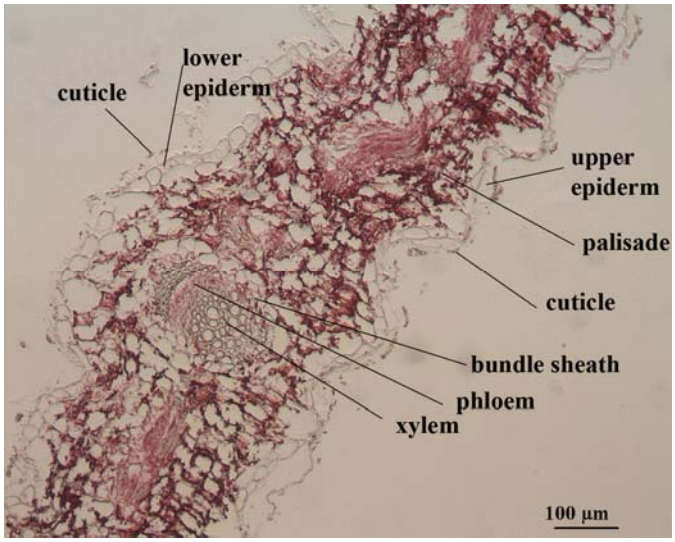

Figure 3.2.8. Cuticle, Lower and Upper Epiderm, Palisade, Bundle Sheath, Phloem and Xylem of the subsidiary vein on the semi leaf of *L. globuliferum* (10X) in cross sections.

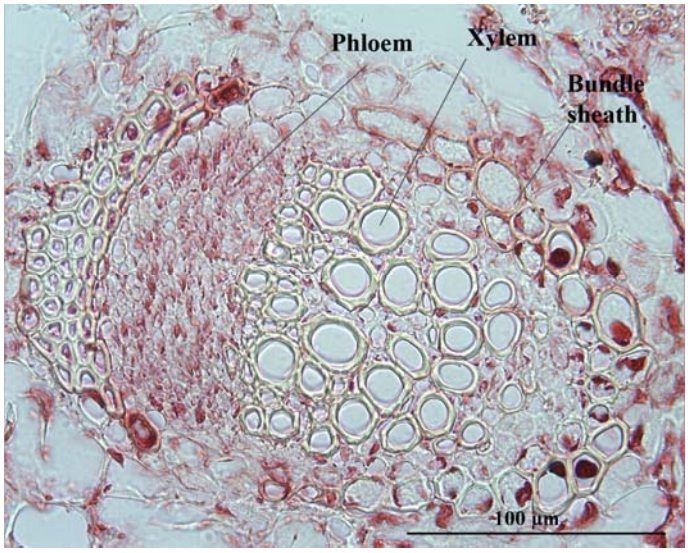

Figure 3.2.9. Phloem, Xylem and Bundle Sheath cells of the main vein of midrib of leaf of *L. globuliferum* (40X) in cross section

At midrib of the leaf, there are four vascular bundles placed and one of them is the main vein which is found in the centre of the semi circle, made up of by the other three smaller and subsidiary veins. Each half of the leaf has a big vascular bundle at the middle from midrib to margins. Away from this, each half of the leaf includes many smaller vascular spaces lined from midrib to margins. The xylem and phloem is covered by the bundle sheath cells and the phloem is placed nearer to lower epidermis than the xylem. The lower and upper epidermis are made up of one layered epidermis cells and have a thick cuticle over them.

3.3. *Limonium tamaricoides*

Figure 3.3. *L.tamaricoides* by Galip Akaydın.

3.3.1. Lower Epidermis

The type of the stomata depending on the epidermal cells are anisocytic. The average number of stomata found per 210.68µm x 263.27µm surface is 2.79 ± 1.08. The average length of stomata is 40.08 ± 3.58 µm. The average width of stomata is 29.02 ± 3.22 µm .

Table 3.13 Length of the stomata of lower epidermis of *L. tamaricoides* leaf in µm

40.98	36.04	36.49	39.67	39.79	42.3	35.45	41.86	42.8
40.73	40	37.37	44.79	42.64	35.5	42.81	43.73	37.21
39.4	39.9	36.35	42.94	42.04	38.07	34.18	44.98	34.01
41.83	42.26	36.2	41.59	32.01	41.54	35.35	42.69	40.16
37.23	39.49	38.3	42.25	44.54	40.89	40.08	43.57	37.04
43.04	44.65	43.68	41.59	38.35	42.39	45.61	44.18	41.24
40.7	38.66	46.01	36.7	40.24	39.52	45.44	38.8	45.66
38.36	39.09	32.19	46.01	35.81	41.63	38.57	49.03	38.74
39.99	43.54	43.94	31.78	40.01	39.84	44.13	41.34	32.03
41.87	39.01	40.97	38.17	37.45	40.71	40.34	39.5	39.05
44.99	39.92	36.64	31.29	41.03	34.93			

Avarage = 40.0771875
Std. Dev. = 3.57705368413873

Table 3.14 Width of the stomata of lower epidermis of *L. tamaricoides* leaf in µm

32.84	28.89	30	26.3	29.26	26.73	27.16	33.03	31.83
27.98	25.21	29.34	30.17	33.44	29.07	35.32	27.65	33.76
30.69	25.2	26.23	29.13	23.53	25.84	32.05	30.73	28.73
30.85	29.9	31	30.56	28.57	27.87	27.64	25.66	31.43
21.84	29.81	35.66	24.39	28.58	27.13	23.09	29.79	31.43
26.54	30.48	31.75	26.73	32.27	26.6	36.87	28.34	30.08
23.47	25.56	25.52	26.51	37.08	27.78	29.86	30.85	30.18
26.53	33.71	30.24	24.09	26.76	25.8	29.86	28.49	27.87
29.37	35.12	26.25	30.55	29.71	27.24	28.17	30.21	23.7
29.08	31.19	26.7	30.02	28.46	30.9	25.75	26.49	30.34
28.05	33.3	26.25	24.83	38.32	27.75	32.5		

Avarage = 29.0245360824742
Std. Dev. = 3.21684143111755

Table 3.15 Number of stomata per 210.68µm x 263.27µm area of *L. tamaricoides* leaf

3	2	4	2	3	2	1	3	2
3	4	2	1	2	3	4	3	3
1	3	3	2	2	2	3	5	5
2	2	5	3	4	3			

Avarage = 2.78787878787879
Std. Dev. = 1.08275042592856

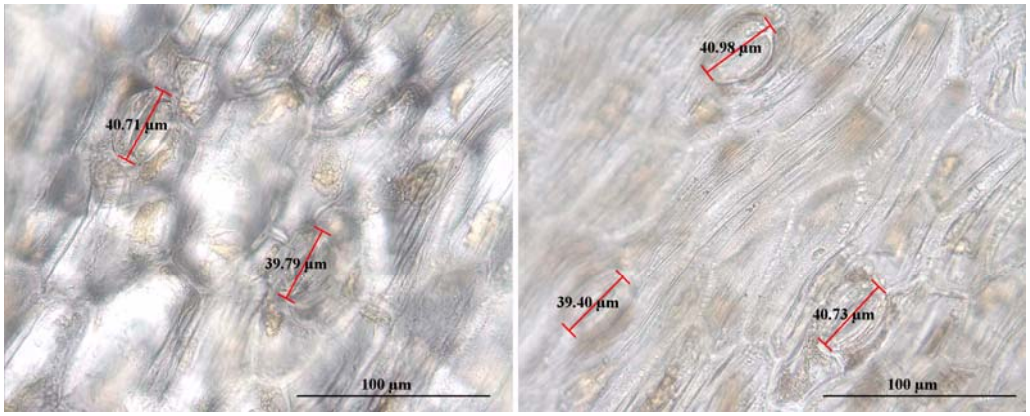

Figure 3.3.1. Photo of two samples of stomatal length of *L. tamaricoides* (40X) (lower epidermis).

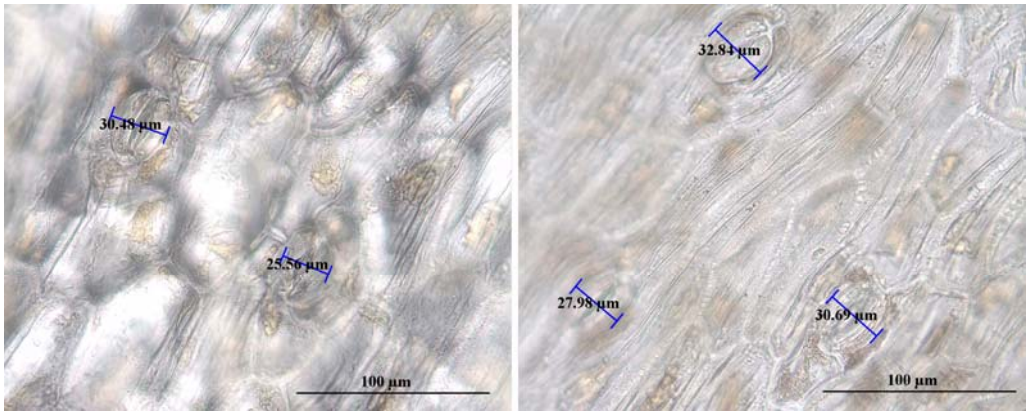

Figure 3.3.2. Photo of two samples of stomatal width of *L. tamaricoides* (40X) (lower epidermis).

3.3.2. Upper Epidermis

The type of the stomata depending on the epidermal cells are anisocytic. The average number of stomata found per 210.68µm x 263.27µm leaf surface is 6.04 ± 1.24. The average length of stomata is 35.89 ± 4.53 µm. The average width of stomata is 29.42 ± 3.10 µm.

Table 3.16 Length of the stomata of upper epidermis of *L. tamaricoides* leaf in µm

25.13	33.63	30.33	42.48	37.16	36.7	36.66	37.6	33.69
42.2	33.35	37.29	33.04	42.83	33.23	35.77	39.89	34.79
36.73	40.25	36.09	35.7	31.74	25.05	33.96	36.92	28.72
37.85	42.52	29.11	37.84	34.88	35.73	34.88	31.77	36.71
38.35	38.99	37	44.54	41.89	33.55	43.24	35.68	37.68
36.1	45.1	33.13	33.76	34.9	37.81	39.4	32.34	35.83
38.84	46.74	42.97	38.7	41.5	39.93	37.43	36.04	29.1
32.04	37.35	29.36	30.06	30.05	28.64	44.24	36.2	39.06
34.95	41.27	30.6	33.07	28.46	37.3	26.77	31.65	34.38
40.51	42.24	38.11	39.34	31.93	31.26	32.17	34.55	30.65
36	42.64	26.8	44.8	29.47	33.27	26.84	33.96	40.55
36.57	39.41	39.28	35.21	39.08	39.08	35.39	34.92	35.25
37.76	40	31.98	34.82	32.99	35.7	35.63	32.42	33.2
42.11	36.8							
Avarage = 35.8899159663866								
Std. Dev. = 4.5282548918717								

Table 3.17 Width of the stomata of upper epidermis of *L. tamaricoides* leaf in µm

25.18	33.03	24.6	31.78	27.78	26.96	28.88	24.52	31.67
27.44	29.33	29.69	26.82	27	29.16	29.74	30.06	33.84
37.72	24.98	22.09	27.03	31.33	27.67	25.87	31.01	35.45
26.36	27.38	31	26.85	27.48	29.94	22.8	28.8	32.37
25.83	24.35	25.41	32.81	24.45	33.18	29.7	22.88	28.6
29.11	34.39	28.4	32.05	23.98	24.73	32.83	29.93	31.39
30.09	29.08	28.9	29.62	29.25	32.63	27.04	32.89	30.54
28.26	30.18	30.03	30.33	27.27	30.64	30.17	27.93	26.61
33.57	27.88	31.81	29.82	28.92	33.88	29.67	30.35	26.83
30.18	30.11	34.06	32.22	30.73	31.01	30.65	31.94	32.19
30.06	28.83	26.59	35.48	36.13	27.13	27.41	31.98	28.14
34.69	26.62	30.54	34.5	30.85	29.79	31.21	31.79	25.18
31.54	29.48	26.85	33.96	29.51	26.53	28	30.56	24.02
34.31	29.75	24.6						
Avarage = 29.4245								
Std. Dev. = 3.09795591323308								

Table 3.18 Number of stomata per 210.68µm x 263.27µm area of *L. tamaricoides* leaf

8	5	5	5	7	8	5	6	5
6	8	7	7	4	4	7	7	6
5	6	6						
Avarage = 6.04761904761905								
Std. Dev. = 1.2440333788203								

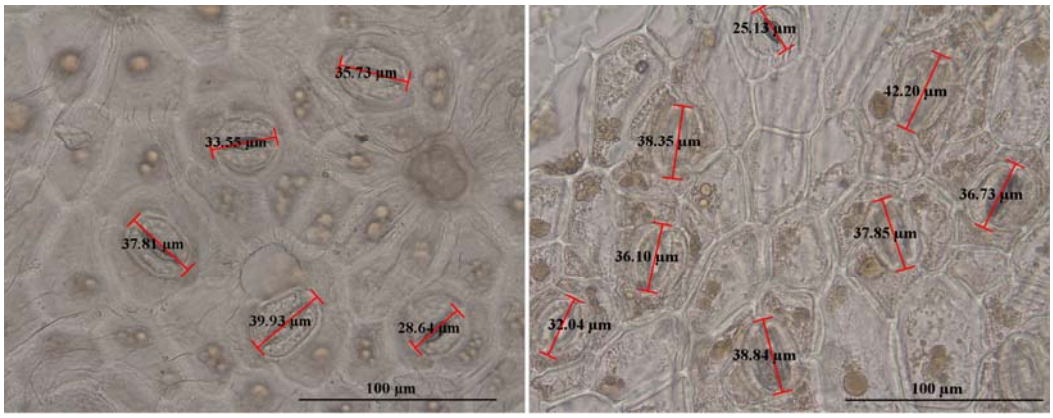

Figure 3.3.3. Photo of two samples of stomatal length of *L. tamaricoides* (40X) (upper epidermis).

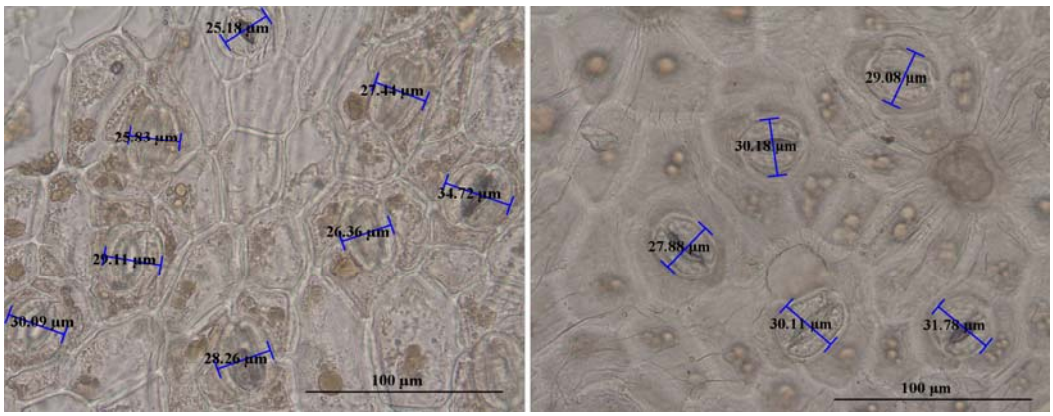

Figure 3.3.4. Photo of two samples of stomatal width of *L. tamaricoides* (40X) (upper epidermis).

Although the average stomatal length and width are almost equal to each other for upper and lower epidermis, the stomatal density per 210.68µm x 263.27µm area of leaf differs for upper and lower epidermis. The upper epidermis again has about 4 units higher for stomatal density than the lower epidermis.

3.3.3. Cross Sectioning

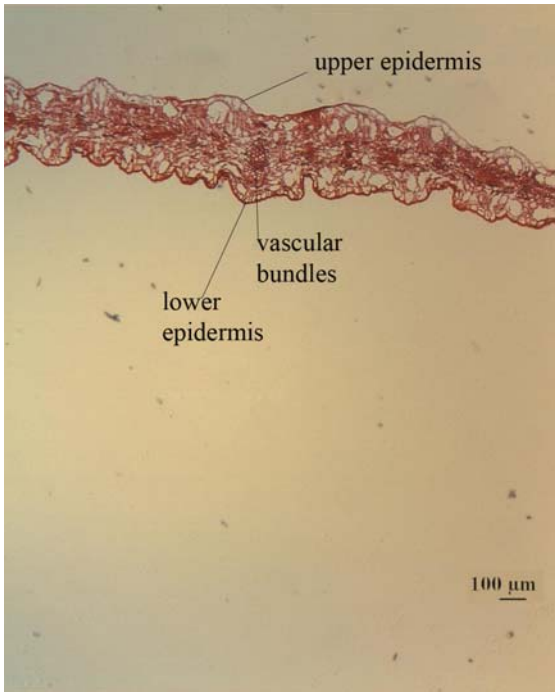

Figure 3.3.5. Lower and Upper epiderm and Midrib of leaf of *L. tamaricoides* (4X) in cross section

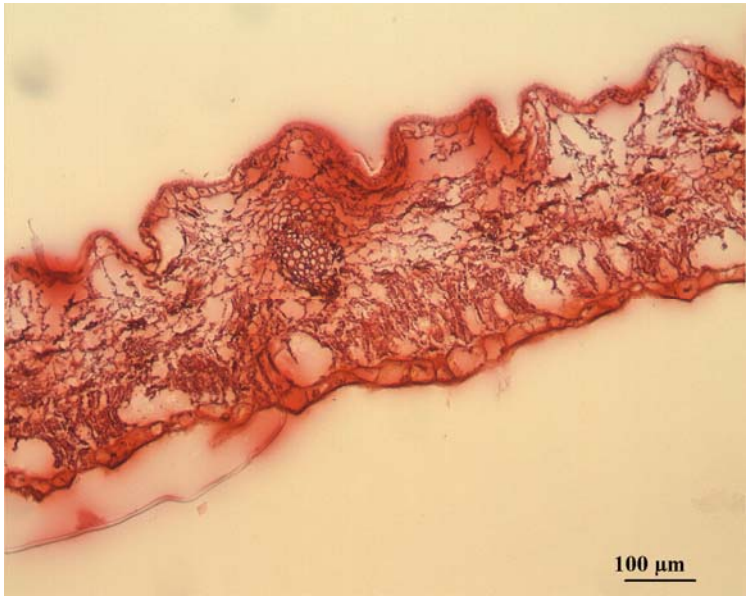

Figure 3.3.6. Microtome cross section of midvein of leaf of *L. tamaricoides* (10 X) in cross section

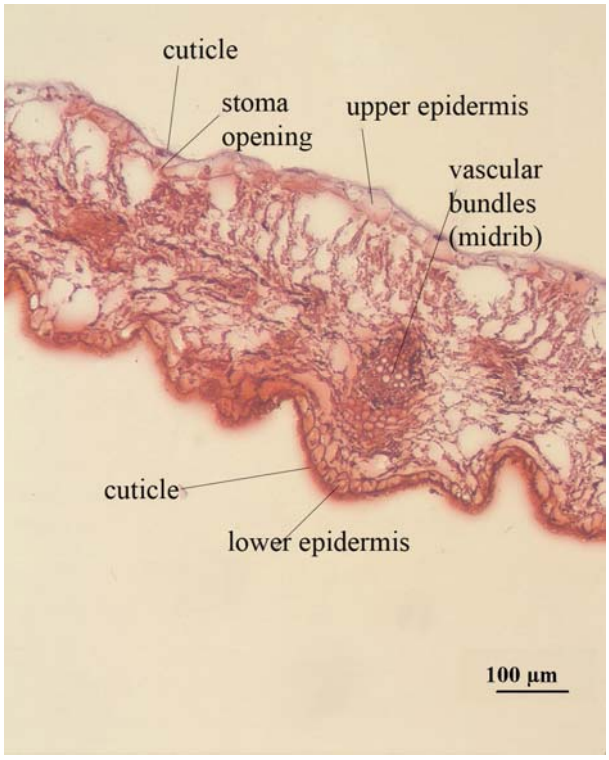

Figure 3.3.7. Lower and Upper Epiderm, Midrib and Stoma opening of leaf of *L. tamaricoides* (10X) in cross section

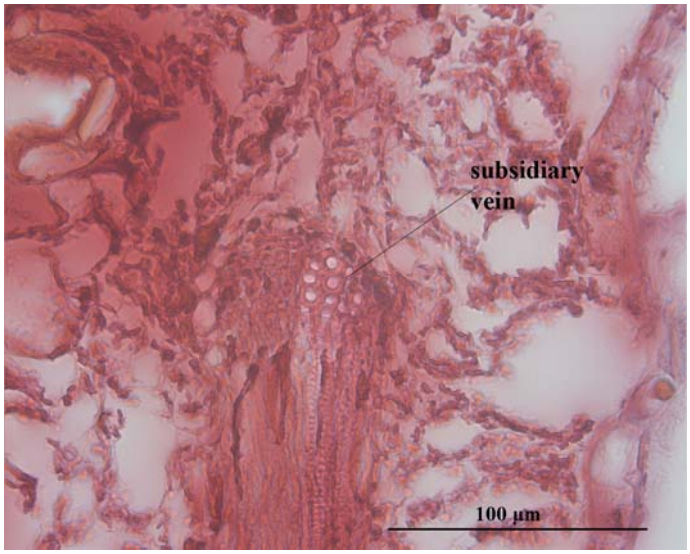

Figure 3.3.8. Subsidiary rib nex to midrib of leaf of *L. tamaricoides* (40X) in cross section

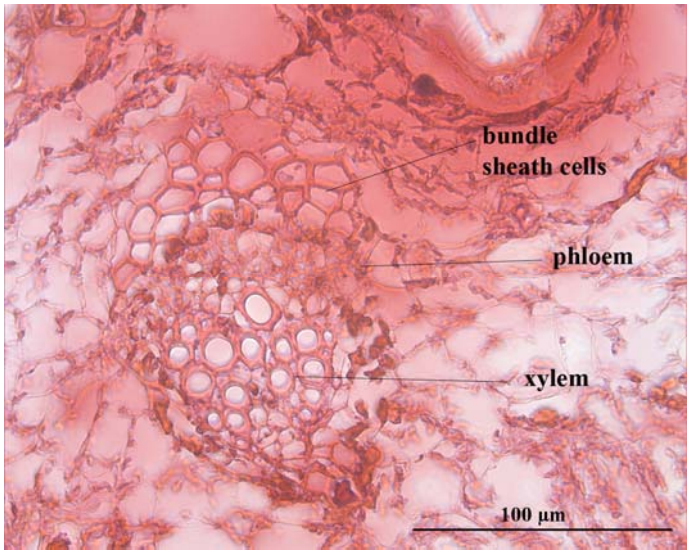

Figure 3.3.9. Xylem, Phloem and Bundle Sheath Cells of midrib of leaf of *L. tamaricoides* (40X) in cross section

L. tamaricoides have many small vascular bundle like structures through the x axis. The midrib and the main vascular bundle is found at the center of this x axis. In the midrib, vascular bundles and the bundle sheath cells are seen easily and the phloem is nearer to lower epidermis than upper epidermis when compared the location of xylem. Also in *L. tamaricoides*, there seen aerenchyma is more condensly distributed through palisade parenchyma than the spongy parenchyma.

3.4. *Limonium anatolicum*

Figure 3.4. *L. anatolicum* by Galip Akaydın

3.4.1. Lower Epidermis

The type of the stomata depending on the epidermal cells is anisocytic. The average number of stomata found per 210.68µm x 263.27µm of leaf surface is 4.23 ± 1.11. The average length of stomata is 34.24 ± 3.36 µm. The average width of stomata is 28.95 ± 2.90 µm.

Table 3.19 Length of the stomata of lower epidermis of *L. anatolicum* leaf in µm

39.05	39.25	34.58	34.22	30.55	33.69	34	31.91	33.95
35.82	32.53	33.78	30.33	33.82	28.31	35.46	34.93	37.08
35.96	36.59	33.69	32.9	41.67	34.76	31.04	31.71	37.56
35.65	29.28	32.25	30.71	36.88	37.84	31.65	33.29	39.09
36.26	27.72	36.18	36.61	29.65	36.48	34.83	34.34	42.63
38.45	40.29	31.75	30.71	34.61	38.94	29.68	34.03	38.97
31.95	36.23	35.32	25.77	33.76	38.04	30.62	36.33	32.39
30.93	35.35	35.53	32.99	41.48	37.84	26.04	33.98	36.61
32.81	33.87	31.33	31.54	34.91	37.25	32.06	29.73	37.58
37.68	34.48	29.54	32.11	31.75	37.66	29.39	33.71	35.7
32.67	35.72	33.61						

Avarage = 34.2383870967742
Std. Dev. = 3.35515284765628

Table 3.20 Width of the stomata of lower epidermis of *L. anatolicum* leaf in µm

27.93	27.39	30.68	31.78	27.82	30.59	31.75	29.17	23.82
29.53	28.48	27.22	29.03	26.68	24.69	34.17	25.94	28.43
28.74	26.29	28.12	32.24	31.86	26.64	31.84	26.79	31.5
27.03	34.39	28.21	26.3	29.75	25.78	33.68	27.58	28.31
28.15	33.95	30.59	26.29	28.35	31.35	29.18	23.2	28.56
30.84	28.02	31.81	26.52	37.36	27.78	26.9	26.84	33.27
30.93	25.19	30.35	31.31	29.3	23.14	29.97	32.24	25.94
29.08	26.62	28.52	34.77	26.21	31.58	33.51	23.76	22.3
28.9	27.71	29.92	32.51	30.36	29.1	28.7	28.51	24.33
27.28	26.56	26.63	31.52	32.97	30.24	31.83	28.4	27.51
31.19	25.68							

Avarage = 28.95304
Std. Dev. = 2.902626

Table 3.21 Number of stomata per 210.68µm x 263.27µm area of *L. anatolicum* leaf

4	4	4	5	5	3	4	7	5
4	4	3	6	3	5	3	5	5
3	5	3	3					

Avarage = 4.227273
Std. Dev. = 1.109776

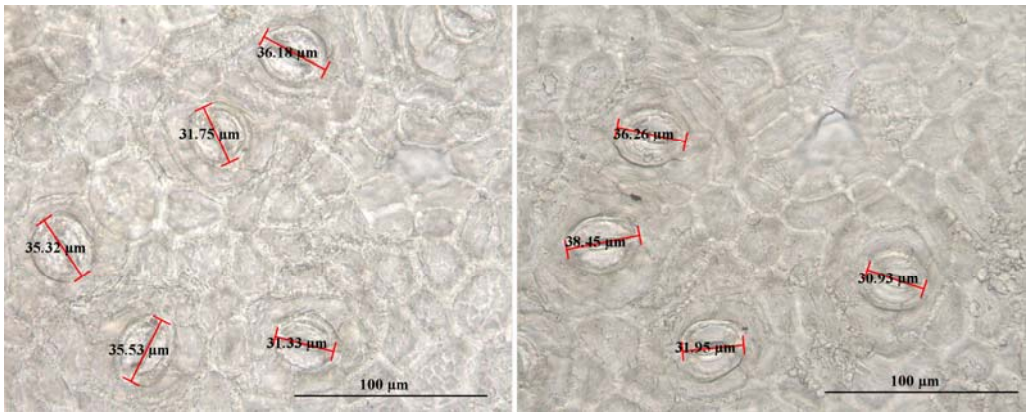

Figure 3.4.1. Photo of two samples of stomatal length of *L. anatolicum* (40X) (lower epidermis).

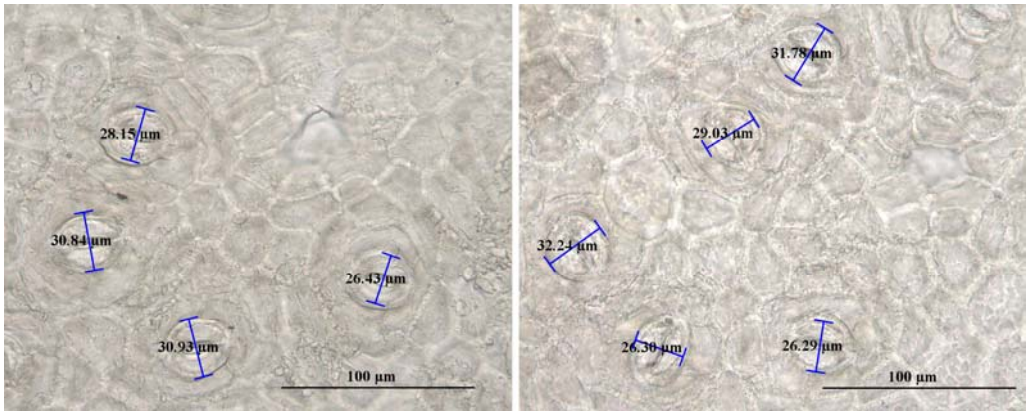

Figure 3.4.2. Photo of two samples of stomatal width of *L. anatolicum* (40X) (lower epidermis).

3.4.2. Upper Epidermis

The type of the stomata depending on the epidermal cells is anisocytic and the average number of stomata found per 210.68µm x 263.27µm of leaf surface is 4.20 ± 0.83. The average length of stomata 33.00 ± 3.24 µm. The average width of stomata is 30.01 ± 4.48 µm.

Table 3.22 Length of the stomata of upper epidermis of *L. anatolicum* leaf in µm

31.42	25.98	40.57	32.44	30.68	35.29	26.39	27.15	34.79
34.06	29.13	35.78	30.73	30.79	35.89	32.68	34.91	35.12
30.62	33.82	34.89	29.37	31.87	32.16	30.79	31.72	39.11
29.53	28.13	33.15	32.74	37.37	35.57	27.32	34.22	34.45
32.97	28.77	39.92	31.72	26.46	37.2	31.99	33.12	36.61
31.19	27.52	28.65	31.67	33.06	37.4	31.58	31.38	34.38
31.59	31.81	32.81	34.65	36.38	35.61	37.05	33.84	32.79
37.6	32.29	32.41	36.75	40.03	34.17	30.64	33.43	36.06
32.87	28.08	32.29	32.28	32.42	31.63	36.11	39.63	33.44
31.5	33.87	31.49						

Avarage = 32.9969047619048
Std. Dev. = 3.23727312595412

Table 3.23 Width of the stomata of upper epidermis of *L. anatolicum* leaf in µm

31.89	28.98	27.76	28.29	29.7	23.77	28.33	32.28	32.3
26.21	25.43	29.67	27.9	34.28	22.12	32.63	30.94	26.63
29.1	27.07	30.43	30.02	30.93	24.47	32.38	30.64	33.8
31.66	26.41	30.43	32.21	33.68	25.25	31.33	28.06	32.9
21.43	26.67	29.55	33.62	31.46	21.13	32.1	30.73	32.94
26.83	33.99	29.18	30.04	25.11	30.21	31.53	33.13	59.48
33.74	31.87	31.53	30.37	25.8	32.11	31.75	27.35	32.4
28.77	31.86	33.54	25.67	28.92	22.84	32.92	31.78	27.81
28.48	29.53	29.94	27.99	26.28	33.48	32.03	32.38	29.13
30.98	30.73	32.81	27.4					

Avarage = 30.01412
Std. Dev. = 4.475714

Table 3.24 Number of stomata per 210.68µm x 263.27µm area of *L. anatolicum* leaf

4	4	5	4	4	4	5	5	5
4	5	4	5	3	2	5	5	3
4	4							

Avarage = 4.2
Std. Dev. = 0.833509

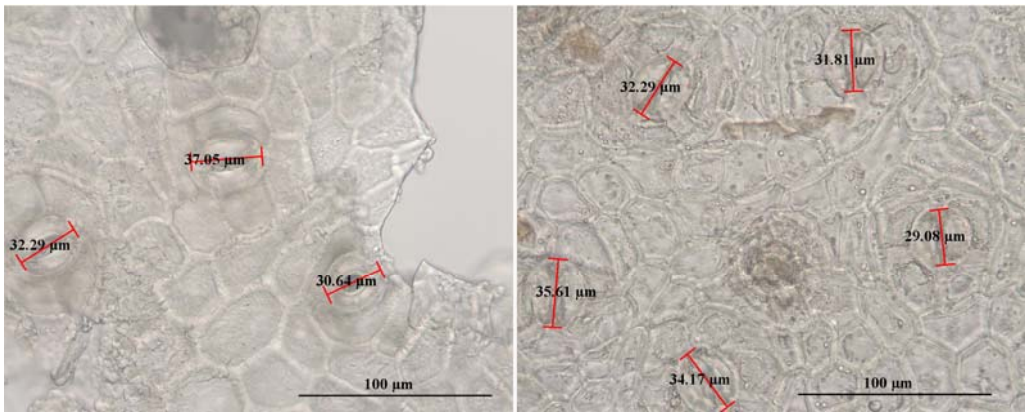

Figure 3.4.3. Photo of two samples of stomatal lenght of *L. anatolicum* (40X) (upper epidermis).

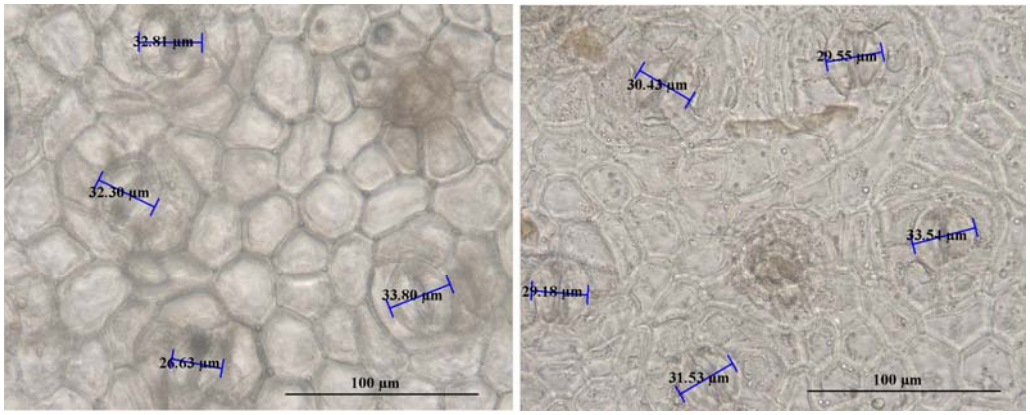

Figure 3.4.4. Photo of two samples of stomatal width of *L. anatolicum* (40X) (upper epidermis).

L. anatolicum, the endemic species, has only the shoot leaves to be examined. And it is calculated that the stomata density per 210.68µm x 263.27µm area of *L. anatolicum* leaf is almost same for upper and lower epidermis of the leaf. In addition, when the stomatal length and width of *L. anatolicum* leaf epidermis is compared, there seen almost no difference between for upper and lower epidermis.

3.4.3. Cross Sectioning

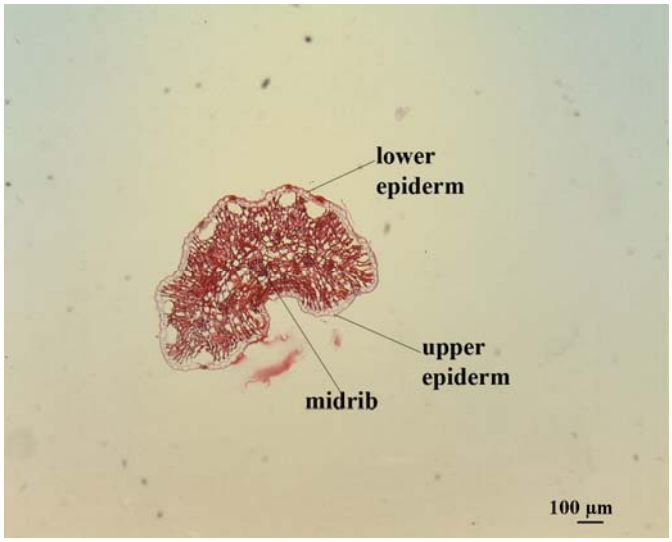

Figure 3.4.5. Upper and Lower epiderm and midrib of leaf of *L. anatolicum* (4X) in cross section

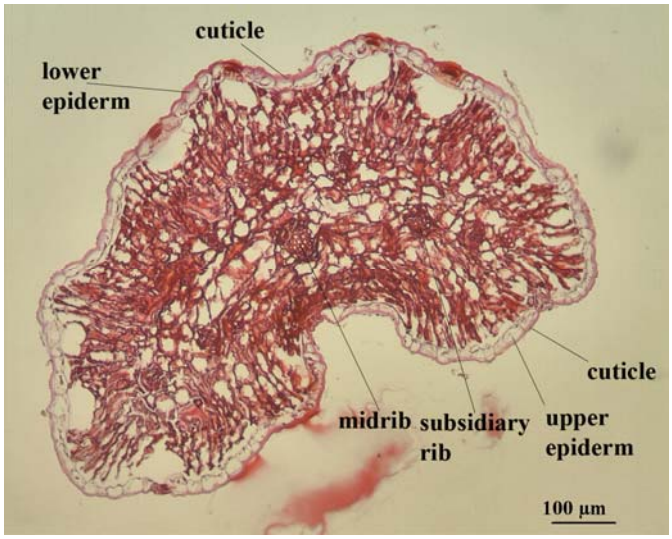

Figure 3.4.6. Midrib, subsidiary rib, cuticle, upper and lower epiderm of leaf of *L. anatolicum* (10X) in cross section

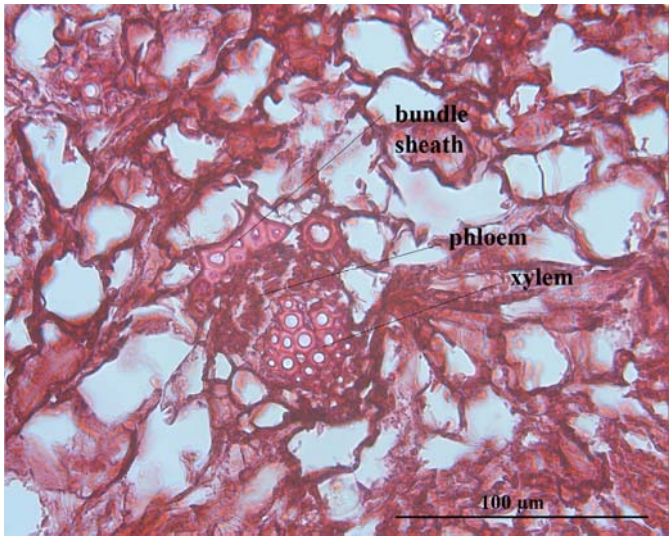

Figure 3.4.7. Phloem, xylem and bundle sheath cells on midrib of leaf of *L. anatolicum* (40X) in cross section

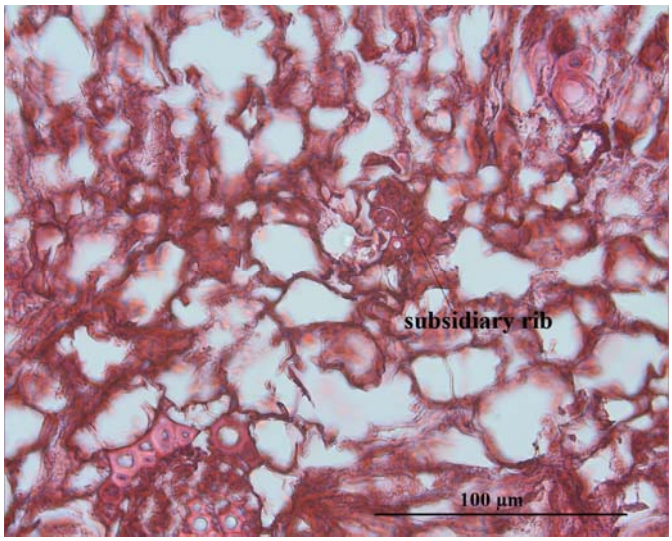

Figure 3.4.8. Subsidiary rib next to midrib of leaf of *L. anatolicum* (40X) in cross section

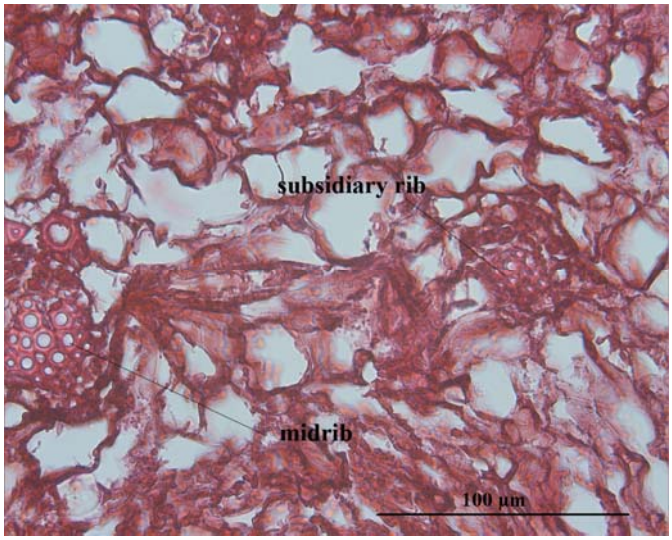

Figure 3.4.9. Other subsidiary rib next to midrib of leaf of *L. anatolicum* (40X) in cross section

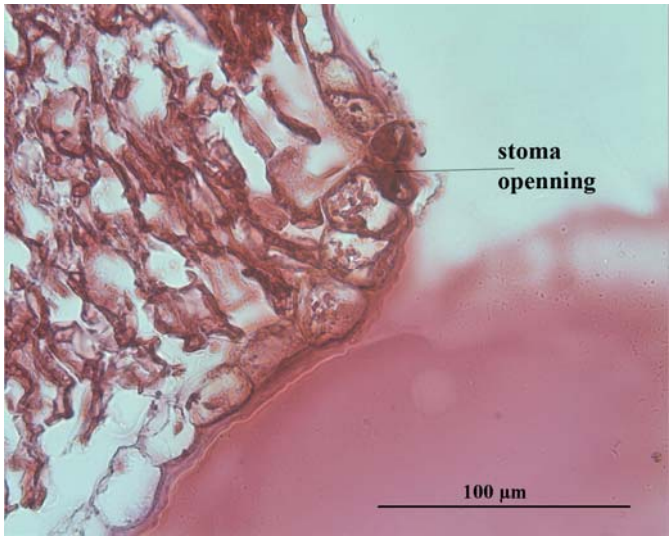

Figure 3.4.10. Stoma opening on upper epidermis of leaf of *L. anatolicum* (40X) in cross section

In *L. anatolicum*, the midrib is visible under 4X magnification easily. The vascular bundles and bundle sheet is seen clearly for the midrib. Also it is again seen that, the phloem is nearer to lower epiderm when compared with the xylem. The aerenchyma, throughout the spongy parenchyma is clearly seen from the photographs and the epidermis layer is single celled for both upper and lower epidermis.

3.5. *Limonium sinuatum*

Figure 3.5 *L. sinuatum* by Galip Akaydın

In this taxa there were two kinds of leaves used. One of the leaf type was basal leaf and the other leaf type was the shoot leaf. Thus throughout the study, for *L. sinuatum*, two different computation were done.

3.5.1. Lower Epidermis

3.5.1.1. Basal Leaf

The type of the stomata depending on the epidermal cells is anisocytic and the average number of stomata found per 210.68µm x 263.27µm of leaf area is 3.22 ± 1.77. The average length of stomata is found as 37.30 ± 5.51 µm . The average width of stomata is 28.77 ± 4.89 µm.

Table 3.25 Length of the stomata of lower epidermis of *L. sinuatum* basal leaf in µm

48.21	28.36	35.44	29.65	37.41	35.82	40.95	39.95	35.72
39.49	29.54	40.41	29.18	35.4	46.73	45.83	39.39	35.14
37.85	33.02	35.22	32.57	38.84	47.13	45.53	40.21	38.84
39.35	32.07	42.07	25.4	39.03	46.9	38.72	44.88	35.34
35.95	30.68	43.47	30.62	43.66	42.64	39.5	36.28	34.06
41.81	32.13	35.96	31.43	35.09	46.75	36.69	34.68	33.67
41.5	30.25	39.47	33.9	36.13	44.24	45.1	35.28	38.39
41.23	25.57	37.62	32.95	35.64	39.77	38.57	40.41	33
34.11	30.11	38.9	28.12	27.39	41.29	38.89	31.47	43.21
36.37	34.47	38.79	25.78	33.96	48.78	37.44	33.28	48.47
38.03	32.83	36.74	30.83	34.8	34.61	44.43	34.15	39.15
41.03	35.45	35.63	35.7	45.32	38.45	38.78	40.24	29.37
45.23	30.28	38.02	34.26	33.5	33.97	24.6	45.72	33.14
44.98	41.83	36.4	29.26	43.9	34.87	41.73	46.04	33.33
44.03	43.75							

Avarage = 37.3038
Std. Dev. = 5.50888

Table 3.26 Width of the stomata of lower epidermis of *L. sinuatum* basal leaf in µm

27.89	29.27	23.2	23.8	35.47	34.03	28.79	24.75	29.19
32.13	30.08	22.17	23.28	22.87	26.62	30.73	24.42	29.31
35.28	30.87	22.62	22.8	24.51	24.57	34.3	23.16	37.04
30.74	29.52	25.8	20.17	23.2	30.18	29.99	24.69	31.54
32.22	33.97	23.76	25.07	26	32.59	25.83	25.32	31.81
33	27.62	24.2	22.89	39	32.96	31.08	30.19	24.91
31.97	30.92	23.7	26	19.61	28.98	28.89	28.32	33.04
36.35	30.83	24.83	21.91	19.33	28.96	26.93	28.41	33.27
31.78	32.74	17.9	23.61	21.05	30.79	24.58	26.23	27.65
34.91	33.09	21.89	20.1	36.64	32.24	22.13	34.81	32.6
32.34	29.63	19.83	20.54	42.17	29.77	20.15	28.36	34.14
35.79	31.33	23.32	32.92	34.95	32.39	35.31	35.12	33.94
30.57	29.74	23.95	28.52	33.54	29.65	33.51	28.92	30.17
29.1	32.53	32.77	24.47	33.6	32.6	34.39	31.23	23.32
30.85								

Avarage = 28.770236
Std. Dev. = 4.8904665

Table 3.27 Number of stomata per 210.68µm x 263.27µm area of *L. sinuatum* basal leaf

1	4	1	6	1	3	6	5	2
1	3	3	5	1	5	5	5	1
1	5	3	4	1	2	5	6	4
4	3	4	2	1				

Avarage = 3.21875
Std. Dev. = 1.77318

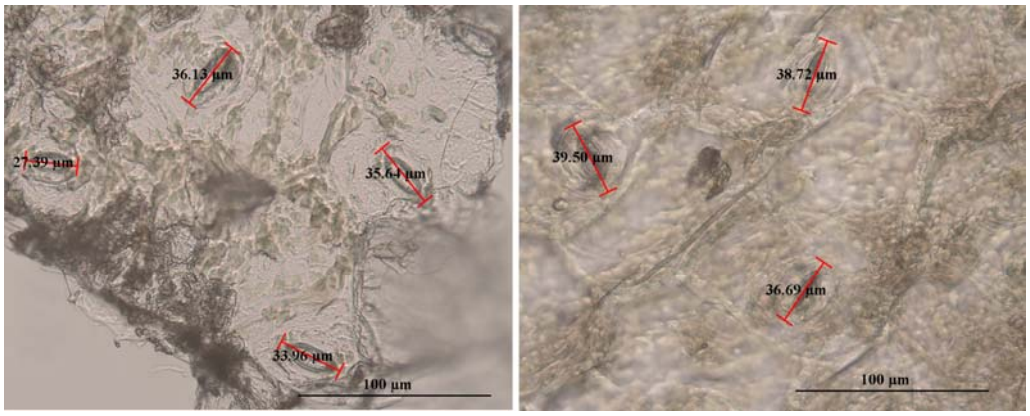

Figure 3.5.1. Photo of two samples of stomatal length of *L. sinuatum* (40X) basal leaf (lower epidermis).

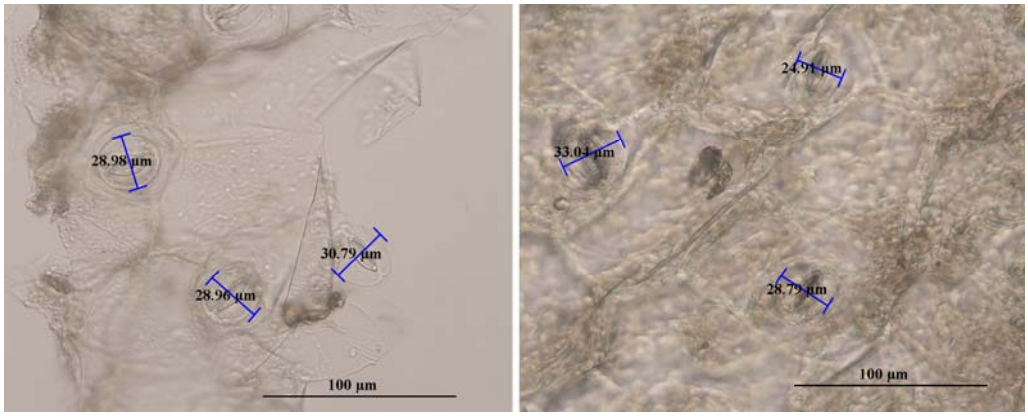

Figure 3.5.2. Photo of two samples of stomatal width of *L. sinuatum* (40X) basal leaf (lower epidermis).

3.5.1.2. Shoot leaf

The type of the stomata depending on the epidermal cells is anisocytic. The average number of stomata counted per 210.68µm x 263.27µm leaf surface is 2.25 ± 1.07. The average length of stomata is 37.13 ± 3.40 µm. The average width of stomata is 27.46 ± 2.78 µm.

Table 3.28 Length of the stomata of lower epidermis of L. sinuatum shoot leaf in µm

39.14	42.95	34.91	39.24	36.4	37.64	39.43	37.69	36.81
37.69	45.12	37.61	36.53	29.43	38.02	33.57	34.11	45.05
32.54	36.46	37.99	37.4	36.29	39.23	34.27	34.45	36.67
31.36	41.3	42.21	35	40.66	38.49	32.27	33.57	36.74
37.5	41.85	39.86	34.15	38.05	35.92	33.22	36.12	33.43
38.58	32.7	45.04	34.72	41.2	33.39	33.05	40.66	35.17
38.46	39.51	38.3	39.37	41.88	37.85	34.56	35.48	39.66
36.47	33.88	37.61	38.25	38.67	29.51			

Avarage = 37.13493
Std. Dev. = 3.404467

Table 3.29 Width of the stomata of lower epidermis of *L. sinuatum* shoot leaf in µm

21.27	28.19	25.33	26.61	24.43	30.5	29.84	29.39	29.25
29.59	26.85	26.73	29.74	22.66	27.64	26.03	28.9	29.71
22.73	28.19	29.65	29.84	22.34	31.52	26.88	28	26.87
23.88	26.86	27.71	32.83	23.36	30.47	27.87	28.39	22.5
27.59	31.4	23.96	25.94	26.15	26.41	30.95	28.61	30.42
26.21	30.66	29.01	23.65	21.59	22.98	27.34	30.09	28.09
30.97	29.34	26.32	23.73	29.36	30.62	27.36	26.84	33.44
25.09	28.24	27.88	27.25	28.59	25.79			

Avarage = 27.45536
Std. Dev. = 2.780517

Table 3.30 Number of stomata per 210.68µm x 263.27µm area of *L. sinuatum* shoot leaf

1	3	2	2	1	3	3	5	3
1	2	2	2	2	5	2	2	1
1	2	2	2	2	3			

Avarage = 2.25
Std. Dev. = 1.073394

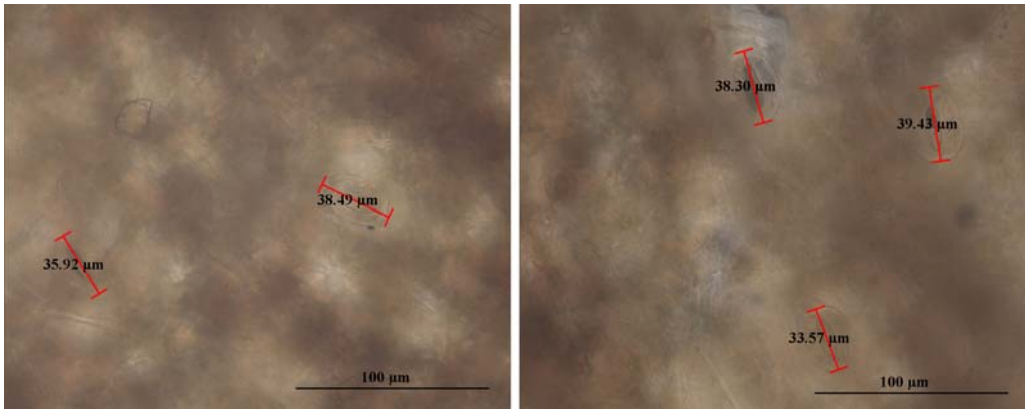

Figure 3.5.3. Photo of two samples of stomatal length of *L. sinuatum* (40X) shoot leaf (lower epidermis).

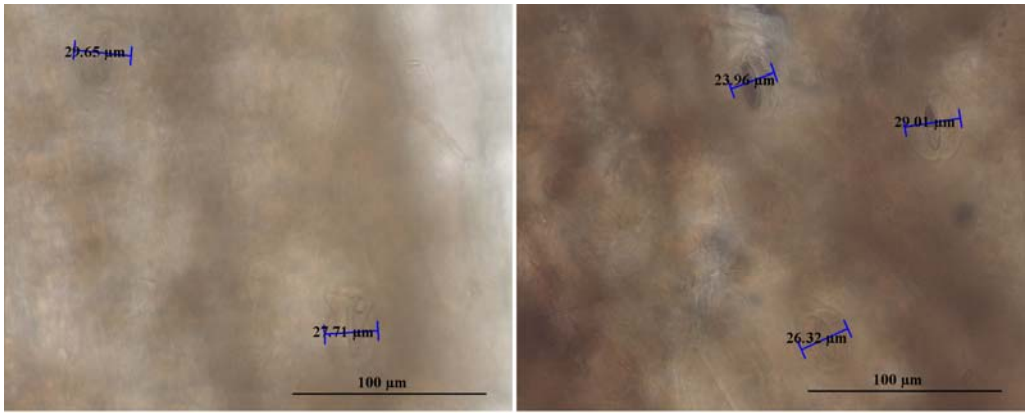

Figure 3.5.4. Photo of two samples of stomatal width of *L. sinuatum* (40X) shoot leaf (lower epidermis).

When the basal and shoot leaf of lower epidermis are compared due to their stomatal length , stomatal width and stomatal density, there is no difference between the shoot and basal leaf of *L. sinuatum*.

3.5.2. Upper Epidermis

3.5.2.1. Basal Leaf

The type of the stomata depending on the epidermal cells is anisocytic. The average number of stomata found per 210.68µm x 263.27µm leaf surface is 2.23 ± 1.57. The average length of stomata is 38.31 ± 6.87 µm. The average width of stomata is 27.69 ± 4.58 µm.

Table 3.31 Length of the stomata of upper epidermis of *L. sinuatum* basal leaf in µm

36.02	37.12	28.25	41.83	30.01	35.74	42.41	45.21	44.03
37.35	45.32	28.01	43.28	40.19	31.06	32.89	40.28	48.11
37.76	37.44	28.34	54.77	25.65	39.6	35.64	41.52	38.35
40.71	46.26	28.86	35.17	25.76	40.65	32.23	36.83	36.27
43.04	40.07	28.31	46.46	25.39	53.18	49.48	39.71	36.35
35.32	37.35	44.79	35.38	31.29	43.6	32.81	44.7	44.03
41.4	48.8	38.92	32.74					
Avarage = 38.31103 Std. Dev. = 6.866286								

Table 3.32 Width of the stomata of upper epidermis of *L. sinuatum* basal leaf in µm

25.46	28.33	27.89	30.54	27.08	21.51	21	28.3	26.27
37.81	31.38	27.14	34.27	29.51	21.73	32.95	29.51	31.84
32.76	21.14	28.71	19.39	25.6	24.3	30.19	23.87	29.62
42.95	22.9	29.17	29.75	26.08	30.34	29.91	29.63	24.43
29.94	31.31	18.57	28.06	24.17	28.4	31.26	27.72	26.48
32.39	30.16	26.83	21.73	29.16	28.64	21.46	25.74	20.03
Avarage = 27.69093 Std. Dev. = 4.583511								

Table 3.33 Number of stomata per 210.68µm x 263.27µm area of *L. sinuatum* basal leaf

1	1	1	8	3	2	2	1	2
3	4	2	1	2	3	1	1	2
2	3	3	1					

Avarage = 2.227273
Std. Dev. = 1.571527

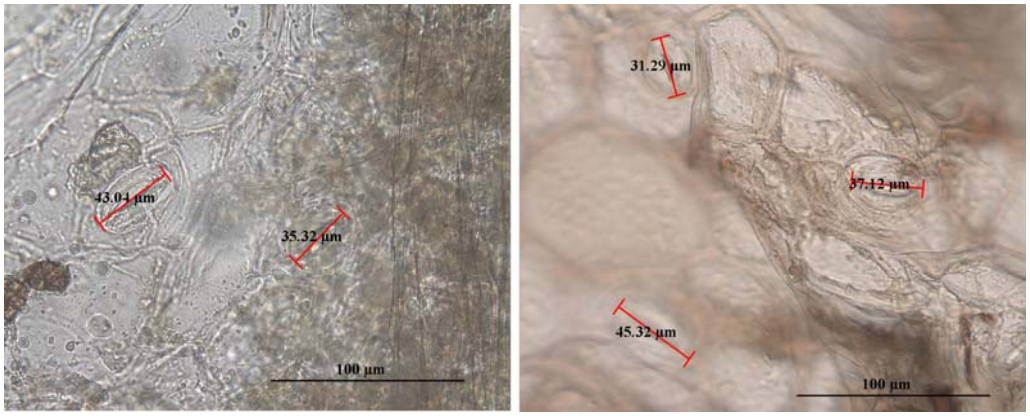

Figure 3.5.5. Photo of two samples of stomatal length of *L. sinuatum* (40X) basal leaf (upper epidermis).

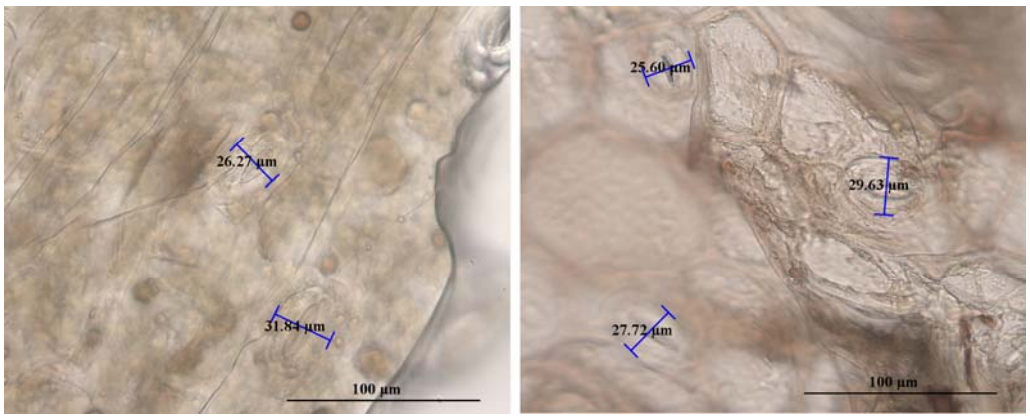

Figure 3.5.6. Photo of two samples of stomatal width of *L. sinuatum* (40X) basal leaf (upper epidermis).

3.5.2.2. Shoot Leaf

The type of the stomata depending on the epidermal cells is anisocytic. The average number of stomata found per 210.68µm x 263.27µm leaf surface is 1.40 ± 0.50. The average length of stomata is 35.66 ± 6.57 µm. The average width of stomata is 23.81 ± 5.25 µm.

Table 3.34 Length of the stomata of upper epidermis of *L. sinuatum* shoot leaf in µm

36.54	32.99	31.4	49.55	29.08	43.71	40.34	31.76	41.72
37.55	40.92	26.98	39.43	32.65	35.3	49.97	30.35	36.08
34.36	31.53	42.96	39.35	27.72	34.04	32.07	36.96	35.63
30.34	38.11	22.49	44	34.15	33.43	43.01	23.35	34.63
28.26	45.38	30.23	45.74	26.58	40.49	46.08	30.24	30.96
31.45	35.93							

Avarage = 35.65511

Std. Dev. = 6.569158

Table 3.35 Width of the stomata of upper epidermis of *L. sinuatum* shoot leaf in µm

19.4	28.77	19.58	22.38	18.57	29.77	28.61	23.32	30.64
20	29.4	16.72	30.51	19.85	33.22	34.65	20.24	29.48
17.1	26.3	17.24	30.96	18.44	29.96	28.61	21.81	25.88
20.05	24.73	13.96	27.28	19.93	23.75	17.57	27.08	22.46
19.98	28.54	17.81	20.53	23.92	21.02			

Avarage = 23.81

Std. Dev. = 5.250082

Table 3.36 Number of stomata per 210.68µm x 263.27µm area of *L. sinuatum* shoot leaf

2	1	1	1	2	1	1	1	1
2	2	2	1	2	1	2	1	2
1	1							

Avarage = 1.4

Std. Dev. = 0.502625

When the basal and the shoot leaves of the upper epidermis of *L. sinuatum* are compared due to stomatal length, width and density; the basal leaf average stomatal length and stomatal width seems to be explicitly higher than the stomatal width and length of the shoot leaf, for upper epidermis.

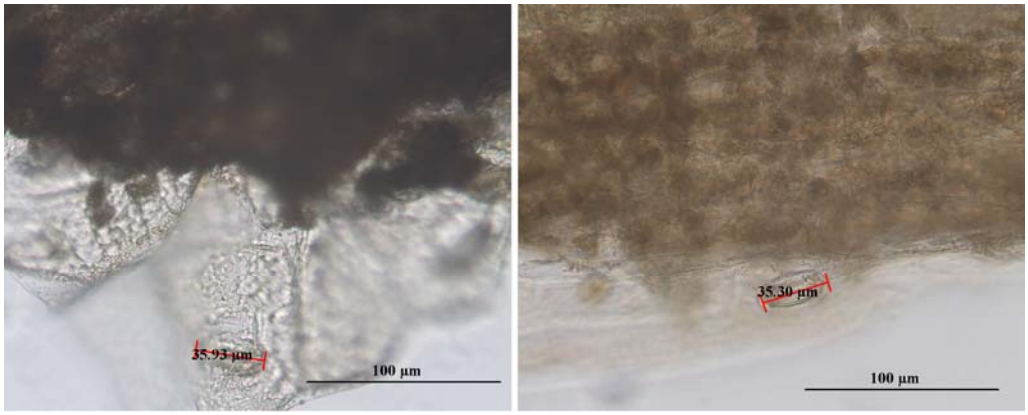

Figure 3.5.7. Photo of two samples of stomatal length of *L. sinuatum* (40X) shoot leaf (upper epidermis).

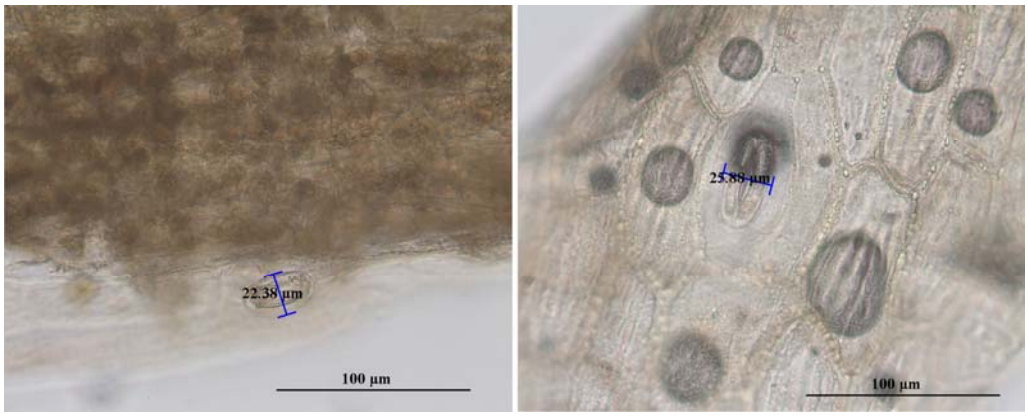

Figure 3.5.8. Photo of two samples of stomatal width of *L. sinuatum* (40X) shoot leaf (upper epidermis).

3.5.3. Cross Sectioning

3.5.3.1. Basal Leaf

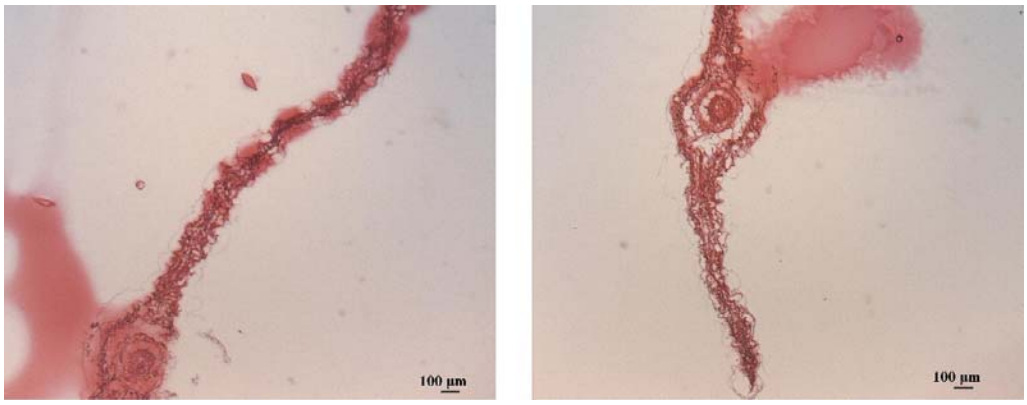

Figure 3.5.9. Basal leaf of *L. sinuatum* (4X) in cross section

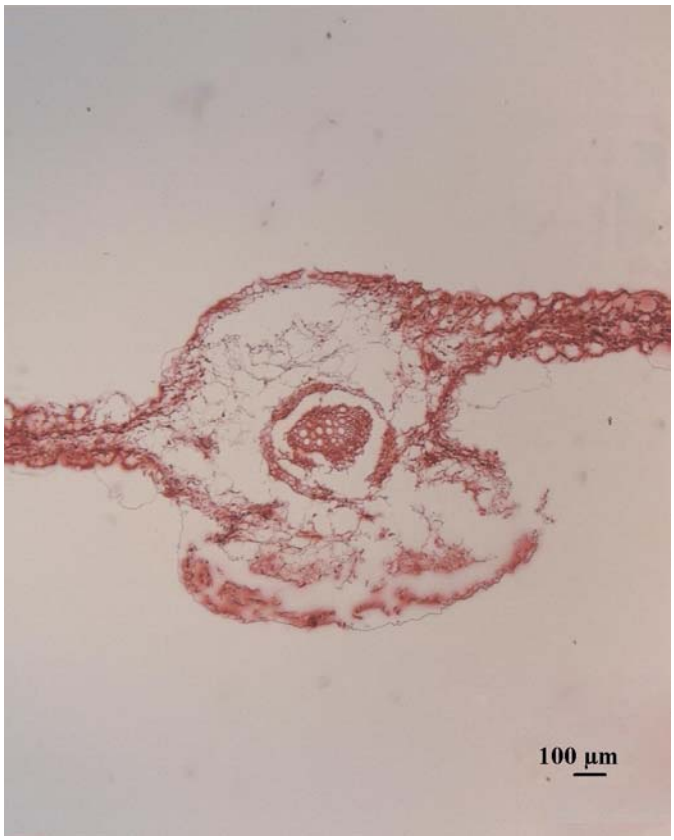

Figure 3.5.10. Basal leaf of *L. sinuatum* (4X) in cross section

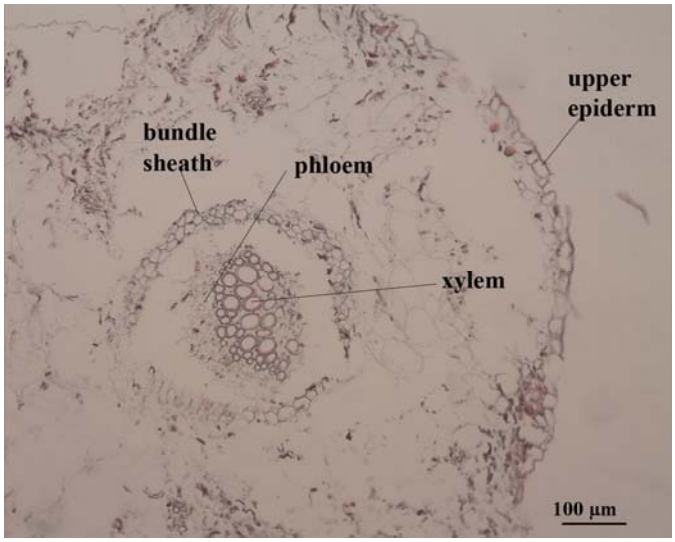

Figure 3.5.11. Xylem, phloem and bundle sheath cells of the midrib of the basal leaf of *L. sinuatum* (10 X) in cross section

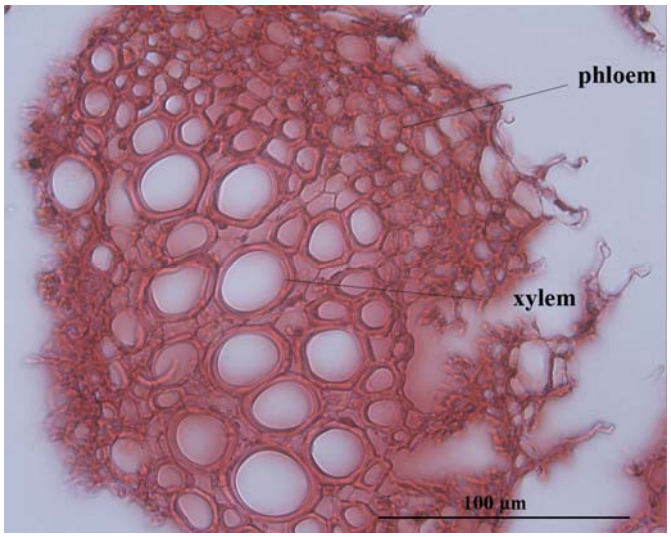

Figure 3.5.12. Phloem and xylem of the midrib of the basal leaf of *L. sinuatum* (40X) in cross section

3.5.3.2. Shoot Leaf

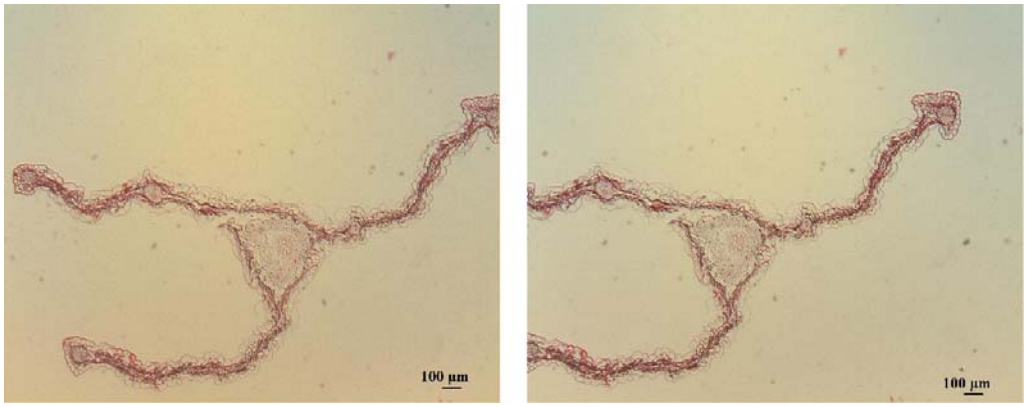

Figure 3.5.13. Shoot leaf of *L. sinuatum* (4X) in cross section

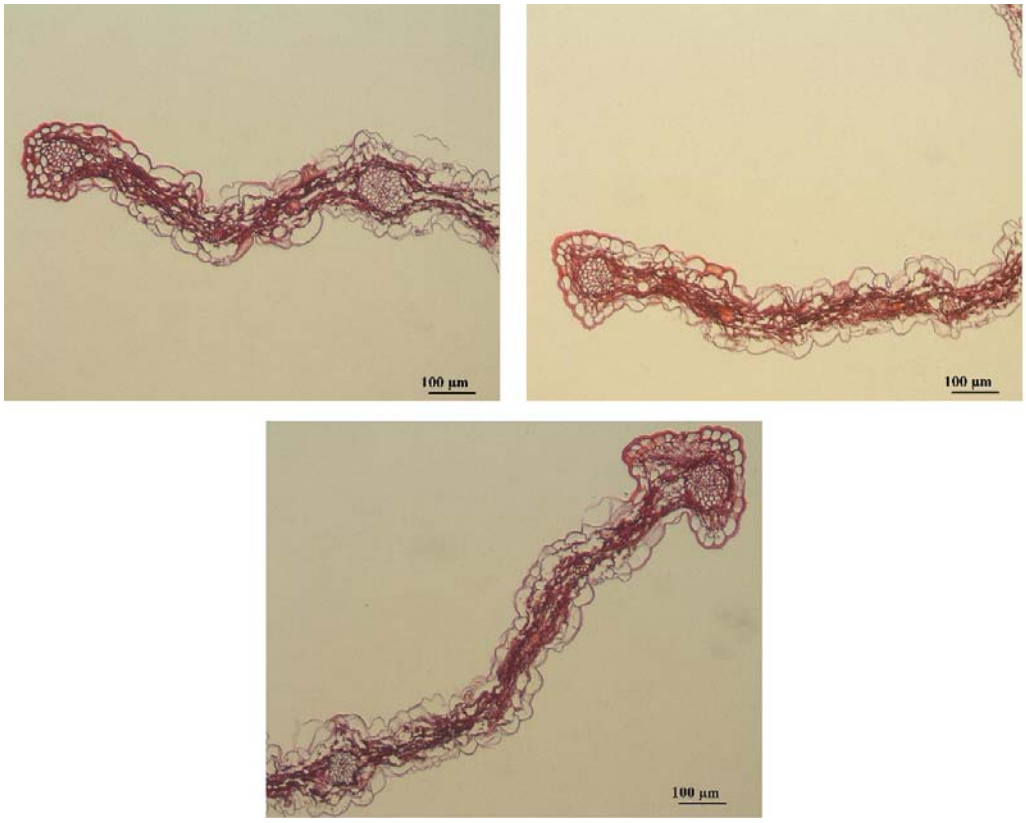

Figure 3.5.14. Three different parts away from the midrib of shoot leaf of *L. sinuatum* (10X) in cross section

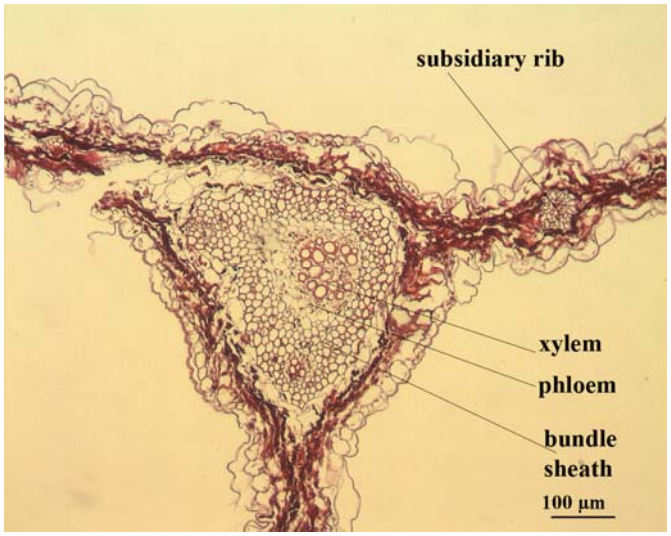

Figure 3.5.15. Subsidiary rib and xylem, phloem and bundle sheath cells of midrib of shoot leaf of *L. sinuatum* (10X) in cross section.

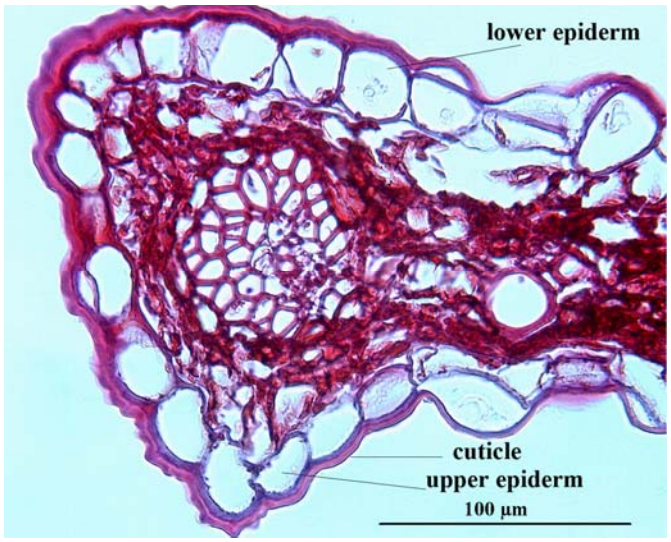

Figure 3.5.16. Lower epiderm, Upper epiderm and Cuticle of one of the end rib of shoot leaf of *L. sinuatum* (40X) in cross section.

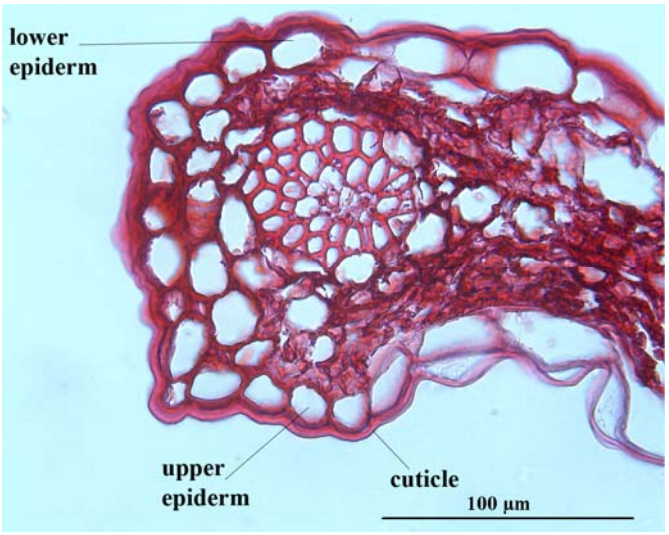

Figure 3.5.17. Lower epiderm, Upper epiderm and Cuticle of one of the end rib of shoot leaf of *L. sinuatum* (40X**)** in cross section.

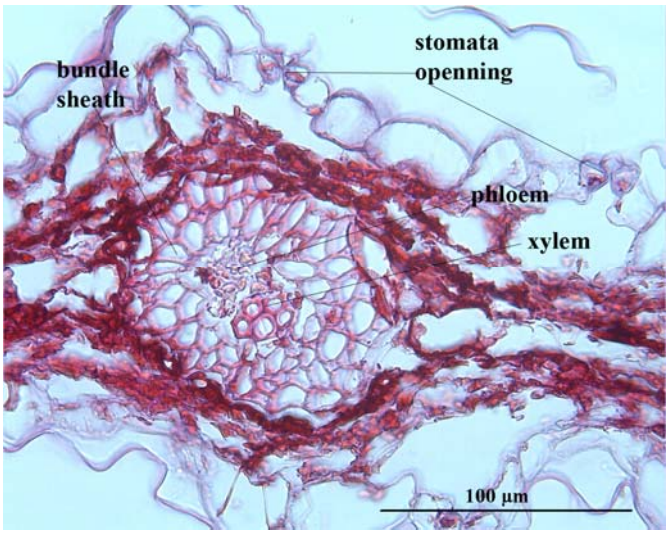

Figure 3.5.18. Bundle sheath cells, phloem, xylem and stomata opening on intermediary rib of shoot leaf of *L. sinuatum* (40X) in cross section.

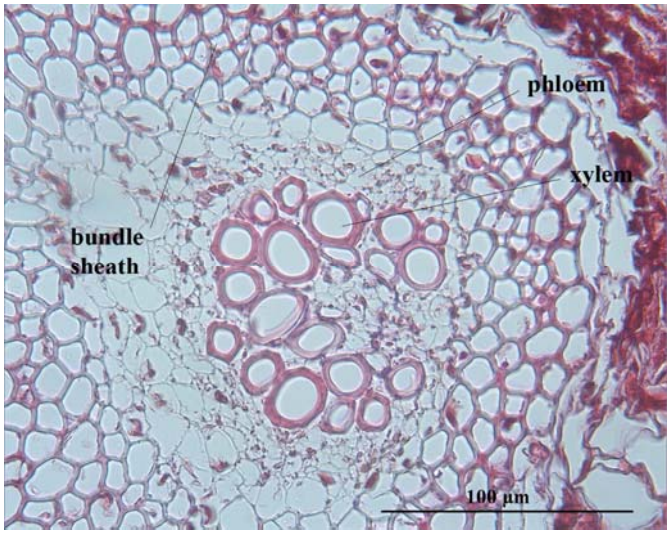

Figure 5.3.19. Xylem, Phloem and Bundle Sheath cells of the midrib of shoot leaf of *L. sinuatum* (40X) in cross section.

Basal leaf of *L. sinuatum* has a wide midrib and different from the other species studied it has no smaller veins additional to main vascular bundle at midrib. The xylem and phloem is circled by the bundle sheath cells but this circle at midrib is not much nearer to the circle made up of phloem and xylem. Similar in structure, it is again the phloem which is nearer to lower epidermis and xylem is positioned nearer to upper epidermis. The subsidiary veins are explicit at the left and right portions of the leaf, from midrib to margins. And their vascular bundles are easily seen and the bundle sheath cells are covering xylem and phloem firmly.

The shoot leaf of *L sinuatum* is exteremly different from the other species of *Limonium* Mill. studied in this thesis. The morphologic structure of the shoot leaf is like a propeller and when the midrib is overlapped with the origin of the x – y axis, the angle between the leaf parts is approximately 120º. Every part of the leaf has one vascular bundle structure at the margins and from midrib to margins, they include one more vascular bundle structure also. The midrib is made up of a huge and sole vascular bundle in which the xylem is circled with a ring like phloem and the bundle sheath cells are around this circle.

CHAPTER 4

DISCUSSION

This study has been conducted in order to examine the leaf anatomy and stomatatypes in five *Limonium* taxa, namely *L. echioides*, *L. globuliferum*, *L. tamaricoides*, *L. anatolicum* and *L. sinuatum*. Among these species *L. tamaricoides* and *L. anatolicum* are endemic and the other species are nonendemic. *L. echioides* is the only annual species. *L. echioides*, *L. globuliferum*, *L. tamaricoides* and *L. sinuatum* have all basal leaves, but *L. anatolicum* is lacking basal leaves and have only shoot leaves.

4.1. Stomatal Features

Table 4.1 Stomatal Features of the lower epidermis of the leaves of the taxa

Name of the Taxon	Stoma Type	Average Stomal Length (µm)	Average Stomal Width (µm)	Average Stomal Density per 210.68µm x 263.27µm leaf surface
L. echioides	anisocytic	30.70 ± 3.94	26.46 ± 2.78	4.05 ± 1.36
L. globuliferum	anisocytic	32.28 ± 3.84	22.88 ± 3.34	3.54 ± 1.14
L. tomoricoides	anisocytic	40.08 ± 3.58	29.02 ± 3.22	2.79 ± 1.08
L. anatolicum	anisocytic	34.24 ± 3.36	28.95 ± 2.90	4.23 ± 1.11
L. sinuatum (basal leaf)	anisocytic	37.30 ± 5.51	28.77 ± 4.89	3.22 ± 1.77
L. sinuatum (shoot leaf)	anisocytic	37.13 ± 3.40	27.46 ± 2.78	2.25 ± 1.07

Table 4.2 Stomatal Features of the upper epidermis of leaves of the taxa.

Name of the Taxon	Stoma Type	Average Stomal Length (μm)	Average Stomal Width (μm)	Average Stomal Density per 210.68μm x 263.27μm leaf surface
L. echioides	anisocytic	29.93 ± 3.72	25.61 ± 3.77	3.86 ± 1.17
L. globuliferum	anisocytic	31.18 ± 3.91	24.95 ± 3.08	6.91 ± 1.95
L. tomoricoides	anisocytic	35.89 ± 4.53	29.42 ± 3.10	6.04 ± 1.24
L. anatolicum	anisocytic	33.00 ± 3.24	30.01 ± 4.48	4.20 ± 0.83
L. sinuatum (basal leaf)	anisocytic	38.31 ± 6.87	27.69 ± 4.58	2.23 ± 1.57
L. sinuatum (shoot leaf)	anisocytic	35.66 ± 6.57	23.81 ± 5.25	1.40 ± 0.50

Throughout the five taxa studied, *L. tamaricoides* has the highest average stomatal length in lower epidermis, which is 40.08μm. The smallest stomatal length, 30.70μm, is seen in *L. echioides* for lower epidermis. The difference in average length of the stomata between these two taxa are approximately 10μm, for lower epidermis. For upper epidermis, this estimation changes since the highest average stomatal length,38.31μm, belongs to basal leaf of *L. sinuatum* while the lowest average stomatal length belongs to shoot leaf of *L. sinuatum*.

When the average stomatal widths of these five taxa are compared, it is seen that the highest value,29.02μm, belongs to L. *tamaricoides* for lower epidermis. However, although *L. tamaricoides* has the highest stomatal width for lower epidermis; *L. anatolicum* has 28.95μm in aveage stomatal width while the basal leaf and shoot leaf of *L. sinuatum* have 27.77μm and 27.46μm in average stomatal width respectively. These are close values to average stomatal width of *L. tamaricoides* in lower epidermis. The lowest average stomatal width,22.88μm, belongs to *L. globuliferum* for the lower epidermis. For the upper epidermis, the highest average stomatal width value,30.01μm, belongs to *L. anatolicum*; *L. tamaricoides* has approximately similar average stomatal width value,29.42μm,

with *L. anatolicum*. The lowest value of average stomatal width ,28,31µm, for upper epidermis belongs to shoot leaf of *L. sinuatum*. Actually, the average stomatal width value of shoot leaf of *L. sinuatum* is almost similar with the average stomatal width values of *L. globuliferum*,24.95µm, and *L. anatolicum*, 25.61µm.

The highest average stomatal density, 4.23 per 210.68µm x 263.27µm area, is observed in *L. anatolicum* for the lower epidermis while the lowest value of average stomatal density in lower epidermis ,2.25 per 210.68µm x 263.27µm area, belongs to the shoot leaf of *L. sinuatum*. In the upper epidermis, *L. tamaricoides* and *L. globuliferum* have the highest average stomatal density values which are 6.04 and 6.91 per 210.68µm x 263.27µm area, respectively. Here, the shoot leaf of *L. sinuatum* has the lowest stomatal density for upper epidermis which is 1.40 per 210.68µm x 263.27µm area.

Throughout the taxa studied *L. echioides* has almost the same values of the average number of stomata, average length and width of the stomata, for both lower and upper epidermis layers of the leaf samples. In addition, like *L. echioides*, the endemic species, *L. anatolicum,* also has almost same values of average stomatal length, average stomatal width and average stomatal density for upper and lower epidermis.

When the average stomatal density of *L. sinuatum* is interpreted, for both the shoot and basal leaves, the upper epidermis layer has one digit lower value than the lower epidermis.

In *L. globuliferum* while the average length and width of the stomata for upper and lower epidermis are almost the same, the average number of stomata per defined leaf surface has a difference in number. While in the lower epidermis of the leaf, there are found approximately 3.5 stomata per 210.68µm x 263.27µm

area, the upper epidermis includes approximately 6.91 stomata per 210.68µm x 263.27µm area of the leaf.

The similar result is valid for the endemic species, *L. tamaricoides*. While the average lenght and width of the stomata are almost same for *L. tamaricoides*, the avarage number per 210.68µm x 263.27µm area of the leaf has approximately 3 unit difference for upper and lower epidermis. While the upper epidermis has approximately 6 stomata per 210.68µm x 263.27µm area of the leaf, the lower epidermis has 2.8 stomata per 210.68µm x 263.27µm area of the leaf. When these information are compared with the stomata density of the other species namely *L. echioides*, *L. anatolicum* and *L. sinuatum*, there are seen no other significant difference between the stomata densities. Actually, Doğan and Akaydın (2006) defined that the habitats of *Limonium tamaricoides* and *Limonium globuliferum* are almost the same and as it is defined in Table 2.1, the distribution areas of these species are very near to each other. As Çağlar and Sütyemez (2004) denoted, the habitat and the ecology of the species are very important and may have some impacts on the stomatal densities, stomatal lenght and stomatal width even within the same species. Thus combined with this knowledge, the reason of observing more stomata on upper epidermis than lower epidermis might be related with these species' occurance in similar habitats. However, the number of stomata and the denstiy of stomata are also related with the microclimate and humidity of the habitat. Stomata density seems higher in lower epidermis, when the habitat is arid and hot. But here an adverse situation is observed, because *L. tamaricoides* and *L. globuliferum* are all Irano-Turanian phytogeographical elements and it is observed that they have more stomata at the upper epidermis than lower epidermis. Again this situation may be related to altitude and climatic properties of Kırşehir, which has a semi-arid habitat and the specimens belonging to these species were collected from 1085 and 1070 m. of height near Seyfe Gölü, respectively. Thus this means the microclimate is not hot and arid but cool and semi-arid thus observing more stomata at the upper epidermis than lower epidermis must be related with this situation.

Evert (2006) defined the stomatal density in two different ways. First, he claimed that the stomatal density is the number of stomata on each epidermal surface of leaf per defined leaf area. If this definition is considered, *L. sinuatum* seems to be the most halophytic species throughout the studied taxa, since it has the lowest values of stomatal density for upper and lower epidermis, when compared with the other species and the lowest number of stomata is mostly distinctive characteristics of the halophytic plants. However, the second definition of stomatal density equals to the number of stomata of lower epidermis over the number of stomata of upper epidermis. Thus if these definition is considered, *L. globuliferum* and *L. tamaricoides* are the most halophytic species throughout the taxa studied. And actually the second formula is more compatible with this study beceuse *L. globuliferum* and *L. tamaricoides* were collected from Kırşehir, near Seyfe Gölü, which is a salty lake. Also the soil type that these two species are living is gypsum rock, which let the plant take calcium ions easily, while the soil type on which *L. sinuatum* grows is the beach rock.

4.2. Leaf Anatomies

L. echioides has an especially thick cuticle layer at the midrib region of lower epidermis. For both the upper and lower epidermis, the epidermal layer is single celled. However, at the lower epidermis of the midrib, there is seen a double layered epidermal cells that surrounds the midvein in a semicircular way. In addition, while the epidermal cells of the upper epidermis are oval shaped, the epidermal cells of the lower epidermis are cubic shaped and uniformly arranged. The thickness of the lower epidermis seems to be thicker than the upper epidermis. The leaves are obovate in their outline and flat shape in samples.

The stomatal opening of *L. echioides* is above the epidermal cells in the lower epidermis. The aerenchyma is not only seen in the lower epidermis but also seen under the upper epidermis. The aerenchyma is locally distributed through the leaf margins. There are regions, from the midrib to the margin extremities, where the

bulk of aerenchyma cells are seen symmetrically at eah part of the leaf. There are also seen individual aerenchyma cells which are distributed more in the upper epidermis than the lower epidermis. In the mesophyll layer, it is not possible to make a distinction between the palisade and spongy parenchyma.
At the midrib, there is a sole main vascular bundle and there are no subsidiary veins around. The subsidiary veins are widespread along the margins. The xylem is located over the phloem and phloem is nearer to lower epidermis than upper epidermis. The bundle sheath parenchyma cells cover the xylem and phloem like a circle.

However, the subsidiary veins that are spreaded along the leaf margins, are not photographed since these vascular structures are seen as drifting lines in the leaf cross sections. This may be related to the specimens dry being. It mustn't be related with the embedding procedure, in which if the specimen wasn't to be embedded directly perpendicular to base, the sectioning procedure would not be well enough. Because, if it would be related with the embedding procedure, at least the midrib would not be seen as the other smaller subsidiary veins and also the microtome samples were taken from different leaf specimens in order to reduce the probability of wrong embedding procedure.

When the microtome samples of *L. globuliferum* are examined, the epidermis is single celled for both the upper and the lower epidermis. The cuticle layer thickness is almost same for both upper and lower epidermis. At the mesophyll layer, the spongy parenchyma and the palisade parenchyma are both easily observed; especially at the midrib region. As getting closer to leaf magrin extremities, the clear distinction of the spongy and the palisade parenchyma are lost. The individual aerenchyma cells are rarely seen under the lower epidermal cells and throughout the leaf sections, there are seen no bulky formation of the aerenchyma. The stomatal openings are at the same level with the epidermal cell layers. The leaves of *L. globuliferum* are large and spatulate in their outline and flat shape in samples.

L. globuliferum is one of the species in which the vascular structures are best seen. At the midrib region, there is seen a midvein, semicircled by three subsidiary veins which are nearer to upper epidermis (Figure 3.2.6). From the midrib to the margin extremities, there are smaller subsidiary veins. In addition to these small subsidiary veins, at the middle of each leaf margin, the larger subsidiary veins are placed. In vascular bundles, the xylem is placed over the phloem and located nearer to upper epidermis. The bundle sheath parenchyma circles the xylem and phloem.

L. tamaricoides, an endemic species, has single layered epidermal cells for both the upper and the lower epidermis. The cells of lower epidermis are smaller than the upper epidermis. The cuticle layer is present for both the upper and the lower epidermis but the cuticle over the lower epidermis is thicker than the cuticle layer of the upper epidermis. The stomata openings are placed at the same level with the epidermis layers. It is not possible to differentiate between the spongy and the palisade parenchyma in the mesophyll layer. The aerenchyma cells are seen in both the upper and the lower epidermis. The aerenchyma cells of the upper epidermis are in circular shape and frequently distributed under the upper epidermis while the aerenchyma cells of lower epidermis are in oval shaped and present almost as a singlefile under the lower epidermis. The leaves of *L. tamaricoides* are spatulate in their shape and appear flat shape in microtome samples.

The midrib of *L. tamaricoides* is not distinct anatomically. The main vascular bundle at the midrib is supported with the other smaller subsidiary vascular bundles along the leaf margins, which are not seen clearly due to their sizes. At the middle of each leaf margin, there is a subsidiary vascular bundle, which is a little bit larger than the other subsidiary veins distributed along the leaf margins. In vascular bundles, the phloem is located under the xylem and placed nearer to lower epidermis than the upper epidermis. Also the bundle sheath cells, circling the xylem and phloem, are easily seen.

L. anatolicum, the other endemic species; has spatulate, tiny and thick leaves which are seen in convolute shape in microtome sections. The epidermal cells are single layered and the upper epidermis is very short in length. The cuticle layer is very thin and the stomata openings are at the same level with the epidermis. It is not possible to make a distinction between the spongy and palisade parencyma due to a bowl like shape of the leaf which is very thick at the middle. The leaf margins are not distinct and the aerenchyma is rarely seen under the lower epidermis only.

The main vein is located at the middle of the bowl like leaf and if it was to put at the gravity center of a rectangle, the other small subsidiary veins would have been located at the corners of that rectangle. The phloem is located under the xylem tissue and it is nearer to the lower epidermis than the upper epidermis. The bundle sheath cells are easily seen and covers the xylem and phloem in circle.

L. sinuatum has two different kinds of leaves. The first group is the basal leaf and the second one is the shoot leaf which has 3 distinct winglike extensions from the midrib of the leaf. In addition to that, *L. sinuatum* is also the sole species which has hairs on the leaves throughout the studied species.

The basal leaves of the *L. sinuatum* are lobed at margins and have involuate shape in microtome sections. The hair types that are seen in basal leaves of *L. sinuatum* are the basic unicellular hairs.

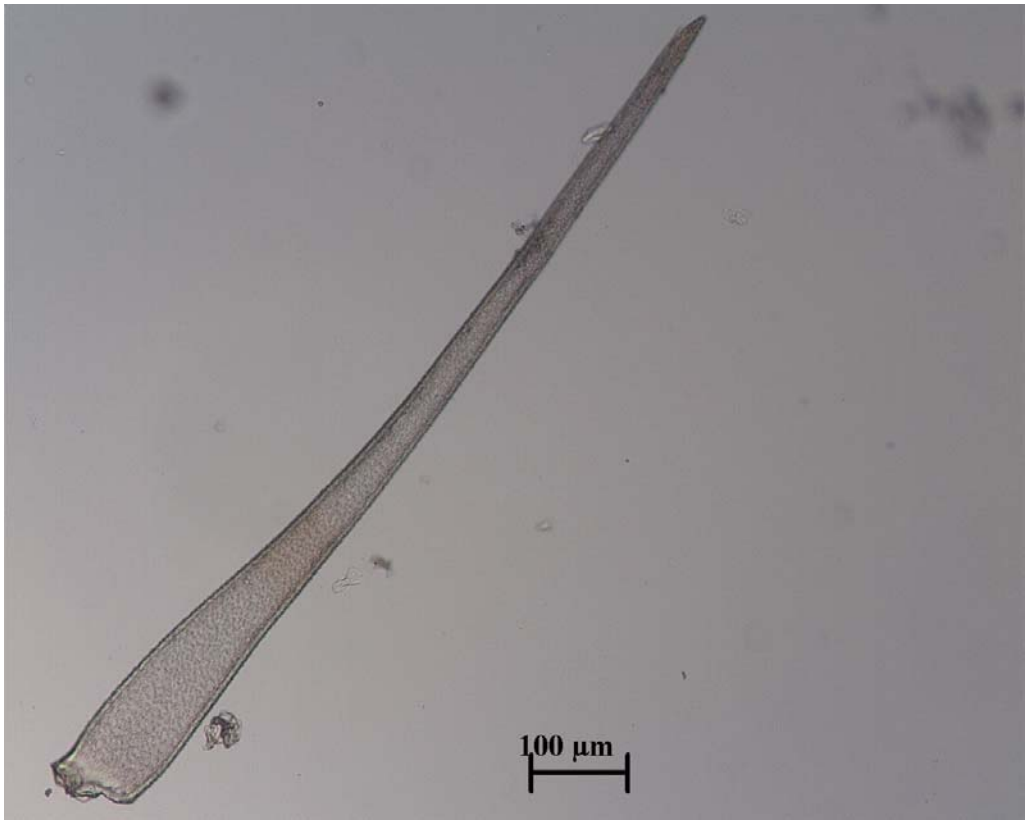

Figure 4.1. A basic unicellular hair sample from *L. sinuatum* leaf.

The upper and lower epidermis are single layered and thick. The cells of the epidermis layers are irregularly shaped. The cuticle layers over them are thin and desultory. The aerenchyma is not frequently seen. The stomata are a little bit over the epidermal layer.

In the mesophyll layer, it is not possible to differentiate between the palisade and spongy parenchyma.

At the midrib, there is a sole, huge vein. While observing from midrib to margin extremities, depending on the leaf part length, there are other distinct subsidiary veins.

For the shoot leaf of *L. sinuatum*, the anatomy of the leaf is somehow more complex. There are irregularyly shaped epidermal cells, covering the whole winglike leaf parts; but these epidermal cells are regularly shaped at margin end points. Though the epidermis is single layered for the both upper and lower

epidermis, at the margin end points, there is seen a multiple layered, semicircle like epidermis, that covers the subsidiary veins found at margin extremities. There is a thin layer of cuticle over the upper and lower epidermis and also there are basic unicellular hairs (Figure 4.1) from epidermal cells. The stomata are at the same level with the epidermis. Also it is not possible to seperate the spongy and palisade tissues at the mesophyll layer.

The midrib is totally made up of the huge main vascular bundle and the phloem circularly covers the xylem tissue. The bundle sheath cells are easily seen. At the edge point of each winglike extension of the leaf, there is a vascular bundle and for each winglike extension, from midrib to edge points, there are seen other three vascular bundles on each leaf part.

When compared with the revisional study (TUBİTAK), conducted by Doğan and Akaydın (2006), the anatomy of the leaves belonging to these five taxa properly suits with the general properties of the genus *Limonium* leaf anatomy, mostly.

Throughout the taxa studied, *L. globuliferum, L. tamaricoides, L. anatolicum* and the basal leaves of *L.* sinuatum have single layered epidermal cells as indicated by Doğan and Akaydın (2006) while *L. echioides* and shoot leaves of *L. sinuatum* have single layered epidermis mostly and double layered epidermis at certain regions of the leaves. Also in this revisional study Doğan and Akaydın (2006), it is featured that in some species of *Limonium,* the mesophyll layer may not be seen with the seperation of palisade and spongy parenchyma. In this study except for the species *L. globuliferum*, the palisade and the spongy parenchyma were not possible to be differentiated in the mesophyll layer. This impossibility may have two reasons. First, it might be related with the anatomical structure of the taxa, as indicated in the revisional study (Doğan & Akaydın, 2006); or it might be related with the specimens' being dry samples. Since the dry specimens are more fragile than the fresh specimens; it is very possible to damage the tissue during microtome sampling.

The genus *Limonium* have vascular bundles in which the phloem is placed under the xylem and located nearer to the lower epidermis than the upper epidermis (Doğan & Akaydın ,2006). Except the shoot leaves of *L. sinuatum*, this structure of vascular bundles are observed in the study. But in the shoot leaves of *L. sinuatum*, the phloem circles the xylem and its closeness is same to the both upper and lower epidermis. Doğan and Akaydın (2006), indicated that the upper epidermis of *Limonium* species are thicker than the lower epidermis. Although the results of *L. globuliferum, L. tamaricoides, L. anatolicum* and *L. sinuatum* shows this indication, *L. echioides* have a thicker lower epidermis than the upper epidermis, partially. Also, except for *L. echioides*, all the species studied, have large subsidiary veins mostly at the middle of the leaf margins but in *L. echioides*, there is seen no larger subsidiary veins at the middle of the leaves but only smaller subsidiary veins spreaded through the margins, only.

Wahid (2003) indicated that the smaller leaves are the morphological adaptations of plants to desert and saline habitats. Due to this knowledge, *Limonium anatolicum*, which is an endemic species and has no basal leaf but only has the shoot leaf that is examined throughout the thesis, has the smallest leaves in morphology, though it is expected to be adapted more saline habitats than other species.

When compared within each other, the studied taxa is divided into two depending on their phytogeography as Mediterrenean species and Irano- Turanian species. *L. sinuatum* and *L. echioides* are the mediterrenean species and *L. globuliferum, L. anatolicum* and *L. tamaricoides* are the Irano-Turanian species. If their rock types are considered, it is seen that the Mediterrenean species are growing on the beach rock which is formed at the places where beach sand dunes are present. At these regions, the lime found in the underground waters cumulates and produces beach rock in the way of attaching sand and coarse grains to each other by crystallization. Then after the loose parts of the composition are carried out by wind or water, there remains the beach rock at the base. The Irano-Turanian

species grows on the gypsum rock which is a type of karstic rock that is found in Central Anatolia. And actually the Irano-Turanian plants takes calcium easily than Mediterrenean plants. Since the calcium is found in the sedimentary rocks as calcium carbonate, it is hard for plants to take calcium that is needed for their structural frames. But gysum is a kind of rock which is made up of by addition of 2 moles of water to calcium sulfate and this gypsum rock type lets plant to take calcium easier. Thus, if the taxa studied compared, it is seen that the Mediterrenean species have multi- layered epidermis partially and the Irano Turanian species shows the general properties of *Limonium* taxa defined in the revision by Doğan & Akaydın (2006). Intercalarily, if the figures 3.2.1, 3.2.2, 3.2.3, 3.2.4, 3.3.3 and 3.3.4 of *L. globuliferum* and *L. tamaricoides* are observed, there is seen almost rounded big dots are easily. These rounded big dots, which are almost at the same size with the stomata around, are the gland surface views which are expected to be observed at the halophyte plants.

CHAPTER 5

CONCLUSION

The leaf anatomies of the taxa *L. echioides, L. globuliferum, L. tamaricoides, L. anatolicum* and *L.* sinuatum haven't been studied before and this is the first study that the leaf anatomies of these five taxa are studied.

Through out these five taxa, *L. anatolicum* and *L. tamaricoides* are the endemic species. *L. echioides* is the sole annual species. Except *L. echioides*, the remained taxa are all perennial.

The leaves studied are the basal leaves for *L. echioides, L. globuliferum, L. tamaricoides* only, while for *L. anatolicum,* the shoot leaves are studied. *L. sinuatum* has two types of leaves as shoot and basal leaves and for *L. sinuatum* both shoot and basal leaves are examined.

Throughout the taxa studied, although *L. globuliferum, L. tamaricoides, L. anatolicum* and the basal leaves of *L. sinuatum* have single layered epidermis, *L. echioides* and the shoot leaves of *L. sinuatum* have both single layered epidermis through the margins of the leaves while they also have multiple layered epidermal cells at certain places. This multiple layered epidermal cells are not suitable with the general leaf anatomy of *Limonium* species.

In addition, *L. echioides* has another diagnostic feature that may separate this taxon from other *Limonium* species. As Doğan and Akaydın (2006) indicated that

the *Limonium* species have a thicker upper epidermis than the lower epidermis. However, *L. echioides* disregards this property of the genus *Limonium* and has a thicker lower epidermis than the upper epidermis.

When the taxa are compared due to the place of stomata on epidermis*; L. echioides* and the basal leaves of *L. sinuatum are* aparted from the other taxa studied as *L. globuliferum, L. anatolicum, L. tamaricoides* and *L. sinuatum.* Because while all these taxa have stomata at the same level with epidermal cells, *L. echioides* has stomata over the epidermal cells and the basal leaves of *L.sinuatum* has stomata a little bit over the epidermal cells. Also, the bulky structure of aerenchyma is only seen in *L. echioides.* The aerenchyma of other taxa are found in individually and mostly distributed through the lower epidermis, while *L. echioides* have the aerenchyma almost at the same densities for the upper and lower epidermis.

For the shoot leaves of *L. sinuatum,* the structure of vascular bundles do not suit to the general vascular bundle structure of *Limonium* species. *Limonium* species have phloem under the xylem and phloem places nearer to the lower epidermis. However, the shoot leaves of *L. sinuatum* have the phloem that circles the xylem which places at the middle of the vascular bundle.

Depending on these differences of *L. echioides* and *L. sinuatum,* these taxa have a probability of differentiating from the genus *Limonium* and this study must continue by observing the root and stem anatomy of these two species in order to separate them from the genus *Limonium.* In addition, since the soil types and and the phytogeography of these species are separated from the other taxa that is studied, the ecology and the geology of these two species also must be considered.

Also, this study must be continued since the taxa *L. echioides*, *L. globuliferum, L. tamaricoides, L. anatolicum* and *L. sinuatum* are all in the IUCN categories thus defining the properties and characteristics of these species are important for their sustainability. Especially the *L. tamaricoides* and *L. anatolicum*, which are both

endemic species, their anatomies haven't been studied before and as Karis (2004) indicated that there seemed a lack of information about the anatomy of the *Limonium sinuatum* for its classification and taxonomy.

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