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Foliar Micromorphology and Anatomy of Five Mediterranean Enclaves in Artvin (Turkey)

Melahat OZCAN[1*], Sumeyye YILMAZ[1]

[1]Artvin Coruh University, Faculty of Forestry, Department of Forest Engineering, Artvin, TURKEY
*Corresponding Author: melahat.ozcan@artvin.edu.tr

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Abstract

Aim of study: We aimed to investigate leaf micromorphology including venation types, and petiole and leaf blade anatomical characteristics of five woody/scrubs Mediterranean enclaves (*Cotinus coggygria* Scop., *Rhus coriaria* L., *Arbutus andrachne* L., *Punica granatum* L. and *Jasminum fruticans* L.) in this study.

Area of study: Artvin Province has three distinct climate types. The species studied were collected from natural habitats in Artvin, where Mediterranean climate type occurs.

Material and methods: Leaves of five species were evaluated micromorphologically and anatomically by using stereomicroscopy, light microscopy and scanning electron microscopy.

Main results: Two species have imparipinnately compound leaves, while three have simple ones. Four venation types were determined in the species. *C. coggygria* has channeled anticlinal walls of adaxial epidermal cells and *A. andrachne* has striate cuticular ornamentation in the abaxial surfaces. Differently from others, *R. coriaria* and *J. fruticans* have glandular trichomes, and *P. granatum* has bicollateral vascular bundle. Secretory canals and druses crystals were detected in some investigated species. Most of the species have hypostomatic leaf type, but *J. fruticans* has amphistomatic one. The highest stomata number and indices per mm^2 were observed in *P. granatum,* while the lowest values were found in *J. fruticans.*

Research highlights: Among the species examined, *Punica granatum* with the highest stomatal number and indices per mm^2, narrowed and deciduous leaf with epicuticular wax composition has probably distinctive adaptive strategies to water deficiency and xerophytic habitats.

Keywords: Leaf, micromorphology, anatomy, venation type, Mediterranean enclave, Turkey

Artvin (Türkiye)'de Beş Akdeniz Enklavının Yaprak Mikromorfolojisi ve Anatomisi

Öz

Çalışmanın amacı: Bu çalışmada, beş odunsu/çalı formu Akdeniz enklavı tür (*Cotinus coggygria* Scop., *Rhus coriaria* L., *Arbutus andrachne* L., *Punica granatum* L. ve *Jasminum fruticans* L.)'ün yaprak lamina ve petiol anatomik özellikleri ile damarlanma tiplerinin de dahil olduğu mikromorfolojilerini araştırmayı amaçladık.

Çalışma alanı: Artvin ili üç farklı iklim tipini içinde barındırmaktadır. Çalışılan türler Akdeniz iklim tipinin hakim olduğu, Artvin iline bağlı doğal alanlardan toplanmıştır.

Materyal ve yöntem: Beş türün yaprakları stereomikroskop, ışık mikroskobu ve taramalı elektron mikroskobu kullanılarak mikromorfolojik ve anatomik açılardan değerlendirildi.

Temel sonuçlar: İki tür imparipinnat bileşik, üç tür ise basit yapraklara sahiptir. Türlerde dört farklı damarlanma tipi tanımlanmıştır. *C. coggygria*'nın adaksiyal epidermis hücre duvarlarının oluklu olduğu ve *A. andrachne*'nin abaksiyal yüzeylerinde çizgili kutikular ornamentasyon tespit edildi. Diğer türlerden farklı olarak *R. coriaria* ve *J. fruticans*'da glandular tüyler ve *P. granatum'*da bikollateral iletim demeti bulunmaktadır. Çalışılan bazı türlerde salgı kanalları ve druz kristalleri gözlendi. Türlerin çoğu hipostomatik yapraklara sahip, bununla beraber *J. fruticans*'nın yaprakları amfistomatiktir. 1 mm^2 de en yüksek stoma sayısı ve stoma indisleri *P. granatum*'da, en düşük değerler ise *J. fruticans*'ta bulundu.

Araştırma vurguları: İncelenen türler arasında *Punica granatum*, 1 mm^2 deki en yüksek stoma sayısı ve stoma indisleri, dar, dökülücü ve epikutikular mumsu içerikli yaprak özellikleriyle, türler arasında su eksikliği ve kurak ortamlara adapta olmada belirgin stratejilere sahiptir.

Anahtar Kelimeler: Yaprak, mikromorfoloji, anatomi, damarlanma tipi, Akdeniz enklavı, Türkiye

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Introduction

During the midterm period in which Turkey has been included, in pleistocene and holocene, distinctive climate changes occured and floristic regions replaced or split off. Consequently, different plant species from different phytogeographical areas mixed with each other and relict species were observed in local zones (Rızvanoğlu, 2003). Plant movement occured in Anatolia during cold and hot climate periods, and this was carried out throughout the Anatolian Diagonale described by Davis et al. (1971). It starts from Gümüşhane-Bayburt Provinces which where behind the North Blacksea Mountains and reach to Toros Mountains in Southwest Anatolia. It has been mentioned that the Anadolu Diagonale as throughout central Toros Mountains reached to the Coruh valley and carried several Mediterranean origin plants to the Blacksea Region and also several colcic origins plants bringed to the Mediterranean Region (Duran & Günek, 2010). During ice age some of them died, but thanks to a temperate microclima present in some valley, the Mediterranean plants have continued their life. If the plants of Mediterranean origin grow in Artvin Province (colchic region), they can be named as Mediterranean enclaves. Especially Hatila Valley and Yusufeli districts of Artvin province have several Mediterranean enclaves.

Artvin Province with altitudes from 250 m to 3940 m, and having three different climates (oceanic, continental and mediterranean), various water supplies, and geological and geomorphological differences has provided special habitats for several plant species (Eminağoğlu et al., 2015). Artvin Province has a total of 2727 taxa belonging to 761 genera and 137 families. 198 taxa of them are endemic and 302 are rare, and totally 500 taxa are under risk. This province has eight different vegetation types and 54% of its covers with woodland. Almost 15 Mediterranean enclaves are present in Artvin. They are as follows; *Olea europaea* L., *Arbutus andrachne* L., *Chamaecytisus hirsutus* Link., *Cistus creticus* L., *C. salviifolius* L., *Cotinus coggygria* Scop., *Rhus coriara* L., *Jasminum fruticans* L., *Ruscus aculeatus* L. *var. angustifolius* Boiss., *Punica granatum* L., *Convolvus cantabrica* L., *Euphorbia peplus* L., *Erodium malacoides* (L.) L'Hér and *Nigella segetalis* M. Bieb. (Eminağoğlu et al., 2015).

Micromorphological patterns such as surface ornamentations and epidermal structures are suggested to be less influenced by the pressures of environmental conditions than other morphological traits (Barthlott, 1981). This is evidence that they are controlled genetically. They have, therefore, been emphasized in taxonomic interpretations in several plant groups (Cutler & Brandham, 1977; Cutler, 1979; Ozcan & Akinci, 2019).

In addition to micromorphological structures, anatomical features, leaves especially are useful at the various taxonomic levels and provide important data for species classification (Metcalfe & Chalk, 1950; 1979). Therefore, the taxonomists have investigated these characters to elucidate taxonomic relationships among the genera or species in recent studies (Stace, 1984; Lu et al., 2008; Inceer & Ozcan, 2011; Eminagaoglu & Ozcan, 2014; Ozcan, et al., 2015). Leaf venation patterns have been also used in species identification and delimitation (Grear, 1970; Teixeira & Gabrielli, 2000).

Klimko et al. (2018) studied leaf micromorphology and anatomy of 8 *Dracaena* Vand. ex L. species and reported that epidermal structures and stomatal characters can supply important values to determine the leaf parts of fossils plants and identify them taxonomically.

Several studies have been carried out on the anatomy of Mediterranean species (Yaltırık, 1967; Lin et al., 1984; El-Oqlah, 1996; Fang-Lan et al., 2005; Al-Saghir et al., 2006; Wannan, 2006; Sargın & Selvi, 2016), and important features in their anatomies have been reported. Muntoreanu et al. (2011) investigated leaf anatomy and micromorphology of *Pilocarpus* Vahl. and related genera, and purposed that characters such as stomata and venation types give significant information in the further phylogenetic studies. Wannan (2006) investigated 81 anatomical and palynological characters of 30 species in the family

Asteraceae, and new subfamily Spondiadioideae was determined. Sargın & Selvi (2016) reported comparative anatomical characteristics of five *Cistus* L. from Southwest Anatolia. Different types of trichomes and crystals were found in the studied species, and shape of petiole and location of stomata in leaf surfaces were determined as important distinct characters among investigated species. Perrotta & Arambarri (2004) studied leaf anatomy of *Schinus longifolius* (Jacq.) Lehm. var. *longifolius* Miq., and anomocytic type stomata, dorsiventral mesophyll and druses crytals scattered to the leaf lamina were reported for this species.

Many studies conducted the leaf anatomies of *Pistacia* species (Yaltırık, 1967; Alyafi, 1978; Lin et al., 1984; Özeker & Misirli, 2001; Ait-Said et al., 2004; Al-Saghir et al., 2006) and SEM microscope was used in a few of them. Belhadj et al. (2007) investigated leaf micromorphological characteristics from seven different populations of *Pistacia atlantica* Desf. They found that epidermal structure and trichome density could be determining characters among the populations. Alyafi (1978) and Belhadj et al. (2007) determined trichome types of *P. atlantica* and *P. cabulica* Stocks. El-Oqlah (1996) reported parallel epidermal striations rising from stomata in *P. lentiscus* L. Al-Saghir et al. (2006) examined leaf anatomies of 15 *Pistacia* species comparatively and reported isobilateral mesophyll for *P. vera*, but dorsiventral ones in the others. In addition, stomatal distributions in leaflets were evaluated and, cuticular structures with trichomes and palisade layers were examined in the genus *Pistacia*. It was determined that *P. vera* has distinctly different leaf structure from the other species. The genus *Pistacia* has xerophytic characters and the species can adapt to dry habitats with developing their palisade tissues. According to palisade tissue, Al-Saghir et al. (2006) suggested that *P. vera* with isobilateral type mesophyll was the primitive among their investigated species, whereas the species with dorsiventral mesophyll were advanced ones.

Fang-Lan, et al. (2005) focused on leaf characters of *Cotinus coggygria* from different altitudes and calculated biological parameters such us lengths and diameters of leaves, stomatal and epidermal cell densities, stomatal size, and water capacity biomass and specific weights. According to their results, plant length, biomass, water included in leaves, stomatal size, epidermal density and leaf specific weight decreased in contrary to higher altitudes.

Perrotta & Arambarri (2004) examined root and leaf micrographic characteristics of *Schinus longifolia* var. *longifolia* (Anacardiaceae). They described distinct leaf traits as the presence and distribution of anomocytic type stomata and ratio of stomatal indices in the adaxial and abaxial surfaces.

In the present study, it was aimed to investigate leaf micromorphological and anatomical characteristics of five Mediterranean enclaves naturally growing in Artvin Province in detail, and to provide additional data which can contribute to the taxonomy of the species. This research is the first focused on Mediterranean enclaves. Investigated characteristics are also discussed with respect to their potential value and in relation to the previous works on the genera or families.

Material and Methods

Plant Sample Collection

We chose five woody/scrubs species which are of Mediterranean origin for this investigation. They were collected from natural habitats in Artvin, Turkey. The species are arranged in alphabetical order and their collections data are listed in Table 1. Specimens for morphological examinations were dried according to standard herbarium techniques and deposited in Artvin Coruh University Herbarium (ARTH).

Table 1. Collection data of investigated species.

Family	Species	Locality	Coordinates	Collection Number
Anacardiaceae	*Cotinus coggygria* Scop.	Artvin; Yusufeli, roadsides, 556 m	40º48'54.8"N, 41º36'18.7"E	02 (S. Yılmaz)
	Rhus coriaria L.	Artvin; Borçka, 10 km to Artvin, woodland, 223 m	41º14'28.0"N, 41º46'35.6"E	10 (S. Yılmaz)
Ericaceae	*Arbutus andrachne* L.	Artvin; Hatila Valley National park road, in forest, 515 m	41º12'35.4"N, 41º47'03.05"E	04 (S. Yılmaz)
Lythraceae	*Punica granatum* L.	Artvin; Ardanuç, roadsides, rocky slopes, 469 m	41º07'41.7"N, 41º03'43.6"E	08 (S. Yılmaz)
Oleaceae	*Jasminum fruticans* L.	Artvin; near university cental campus, above slopes, among grasses, 240 m	41º11'10.0"N, 41º49'44.2"E	954 (M. Ozcan)

Micromorphological Analysis

Micromorphological features of the completely dried and mature leaves were studied using a stereomicroscope (Leica M60 with a digital camera attachment DFC 295) and a scanning electron microscope (Zeiss Evo LS 10). To determine leaf shapes, color, trichome density, and venation types, stereomicroscope was used. Small parts of leaf midrib, lamina and margins from upper and lower surfaces were separately placed on stubs using double-sided adhesive tape, coated with gold for 2 min (Cressington sputter coater 108 auto coating apparatus), and observed using SEM microscope at an acceleration voltage of 10 kV. All parts including midrib, lamina, margins both of upper and lower surfaces of the species were examined and micrographs were taken from the same region. Micromorphological observations include shapes of epidermal cells, stomatal type and distribution, trichomes, sculpture of cuticle and wax occurrence.

The terminology of the leaf characters proposed by Barthlott (1981) is used to describe cell arrangements and surface ornamentation. Venation types are classified according to Hickey's (1973) and Hickey & Wolfe's (1975) proposals.

Anatomical Preparations

Anatomical observations were performed in the petiole and leaf lamina of the species. Fresh leaf materials were fixed in the field with formalin-acetic acid–alcohol (FAA) for 24 h and stored in 70% alcohol. Handmade cross-sections were prepared from the median parts of the petioles. Transverse sections and peripheral sections of leaves were obtained by hand with the aid of razor blades, and stained in safranine-alcian blue (for cross sections: 1 min in safranine, and, 1 min and 30 second in alcian blue) and haematoxylin (for peripheral sections: about 15 min) solutions. Excess staining were removed from the sections by washing in water several times (Algan, 1981). Permanent and semi-permanent slides were mounted in entellan and glycerine, respectively (Algan, 1981; Vardar, 1987). Slides were examined under a light microscopy and the images were captured using an Olympus BX 53 research microscobe with digital camera attachment DP 73.

Several cross sections were obtained from different individuals of the species to assess the consistency of anatomical characters and to calculate the means and standard error among different cross-sections. Totally ten paradermal slides (five from adaxial and five from abaxial surfaces) were prepared for each species and 50 stomatal lengths were measured on each slide. Stomata were classified in the proposal of Metcalfe & Chalk (1950, 1979) and the stomatal indices were calculated according to the method described by Meidner & Mansfield (1968).

Results

Anatomical features included petiole and lamina observed through light microscopy are summarized in Tables 2 and 3, as well as stereophotomicrographs in Figure 1, SEM micrographs in Figures 2-6 and anatomical structures in Figures 7-11, respectively showing distinct features among the species. The main leaf characteristics of examined species are highlighted in Table 4. Leaf micromorphological characteristics of *C. coggygria* and *R. coriaria*, and anatomical descriptions and measurements of all species were presented here for the first time, in detail. More specific interpretations and illustrations on the following characteristics are described below.

Micromorphology

The species investigated have deciduous leaves, except for *A. andrachne* and *J. fruticans*. The leaves are simple in three of the taxa, while imparipinnately compound in *R. coriaria* and *J. fruticans*. Their shapes are obovate-elliptic in *C. coggygria* with entire margins, oblong leaflets with serrate margins in *R. coriaria*, widely oblong in *A. andrachne*, narrowly oblong in *P. granatum* and narrowly oblong leaflets with entire margins in *J. fruticans*. *C. coggygria*, *P. granatum* and *J. fruticans* have truncate leaf/leaflet tips, while the others have acute ones. Leaf surfaces of *C. coggygria* and *P. granatum* do not have any trichomes, whereas *R. coriaria* and *J. fruticans* have both egladular and glandular trichomes of the capitate and clavate types. Glandular trichomes are evident only on the abaxial surface of *R. coriaria* and on the adaxial surface of *J. fruticans*. *J. fruticans* has also short unicellular trichomes in the leaf margins and both long eglandular trichomes and papillar structures on the lower surfaces. Only sparsely-simple trichomes occur in *A. andrachne*. In addition, the cuticle of *J. fruticans* has papillar structures on the lower leaf midrib, and simple and short rigid trichomes distributed near the leaflets margins. The species without trichomes; *C. coggygria* and *P. granatum*, and partly *A. andrachne* have waxy compound in their leaf surfaces, differently from the other (Figures 2-6).

Leaf surfaces of all species have undulate-ruminate ornamentation. Anticlinal walls are channeled (sunken), while periclinal ones are raised/convex in *C. coggygria*, in contrary to *R. coriaria*. Wavy/undulate periclinal surfaces are observed in the other investigated species, especially in *J. fruticans*. Anticlinal walls of *A. andrachne*, *P. granatum* and *J. fruticans* do not distinctly visible. In the abaxial surfaces, midrib region of all species are sulcate pattern. *A. andrachne* has remarkable striate ornamentation in the lower leaf blade, unlike in the others. Three out of five species investigated have druses crystals in leaf surfaces, but *A. andrachne* and *J. fruticans* do not have. Two species in the family Anacardiaceae; *C. coggygria* and *R. coriaria* have craspedodromous venation in which secondary and tertiary veins reach leaf margins. *A. andrachne* has camptodromous-cladodromous type in which secondary veins are gradually diminish toward to margins and not terminating in there and, *P. granatum* has camptodromous - brochidodromous ones in which secondary veins are gradually diminish toward to margins and curved upward and joined together. In *J. fruticans*, however, hyphodromous venation is observed. In this type, secondary veins are not present or visible because of concealed inside the fleshy leaf blades.

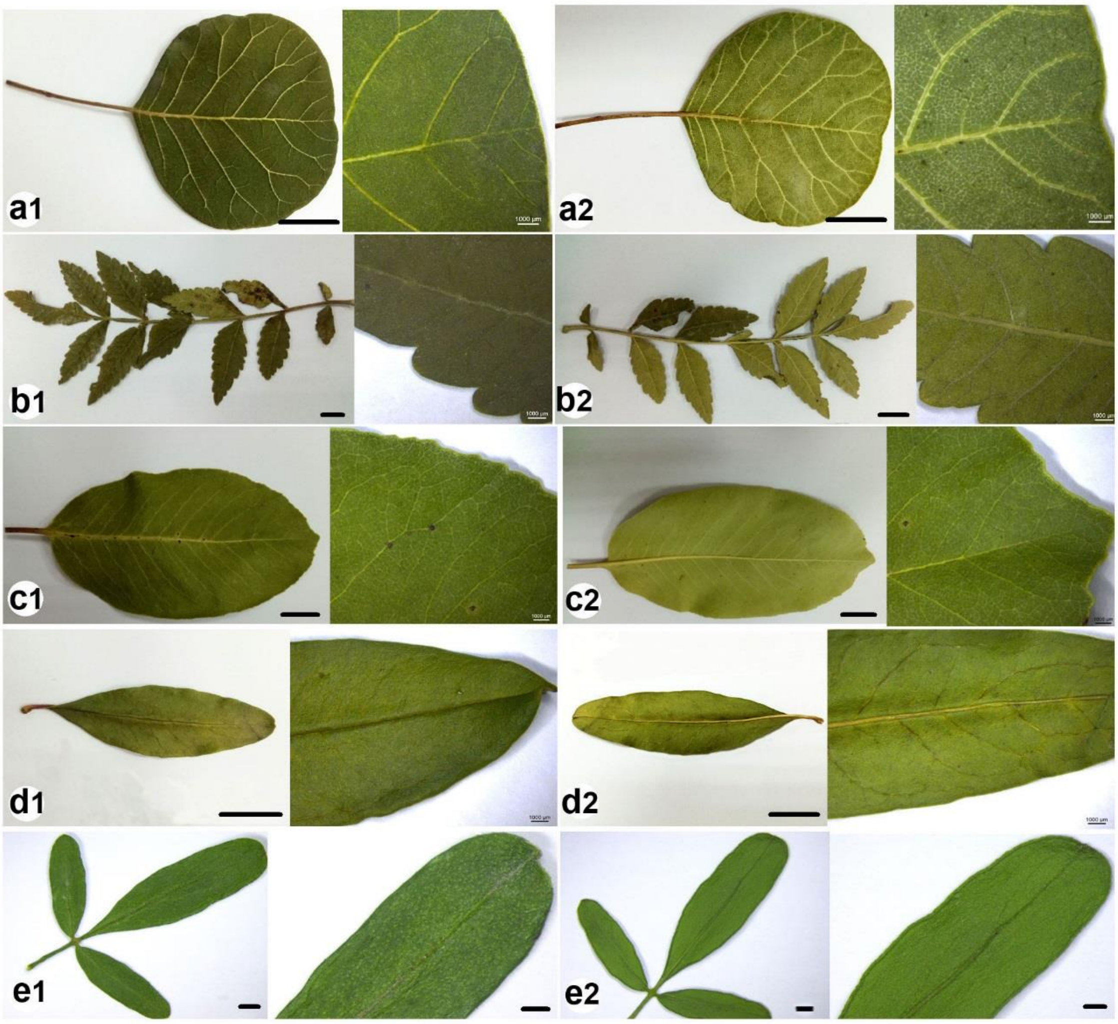

Figure 1. Stereomicrographs of leaves. simple leaf (a, c, d); compound leaves (b, e). a: *Cotinus coggygria*, b: *Rhus coriaria*, c: *Arbutus andrachne*, d: *Punica granatum*, e: *Jasminum fruticans*. 1: adaxial surface, 2: abaxial surface. Scale bars: 1 cm (a-d; 1, 2; left); 1000 µm (a-d; 1, 2; right). e_1, e_2: 1000 µm (left), 500 µm (right).

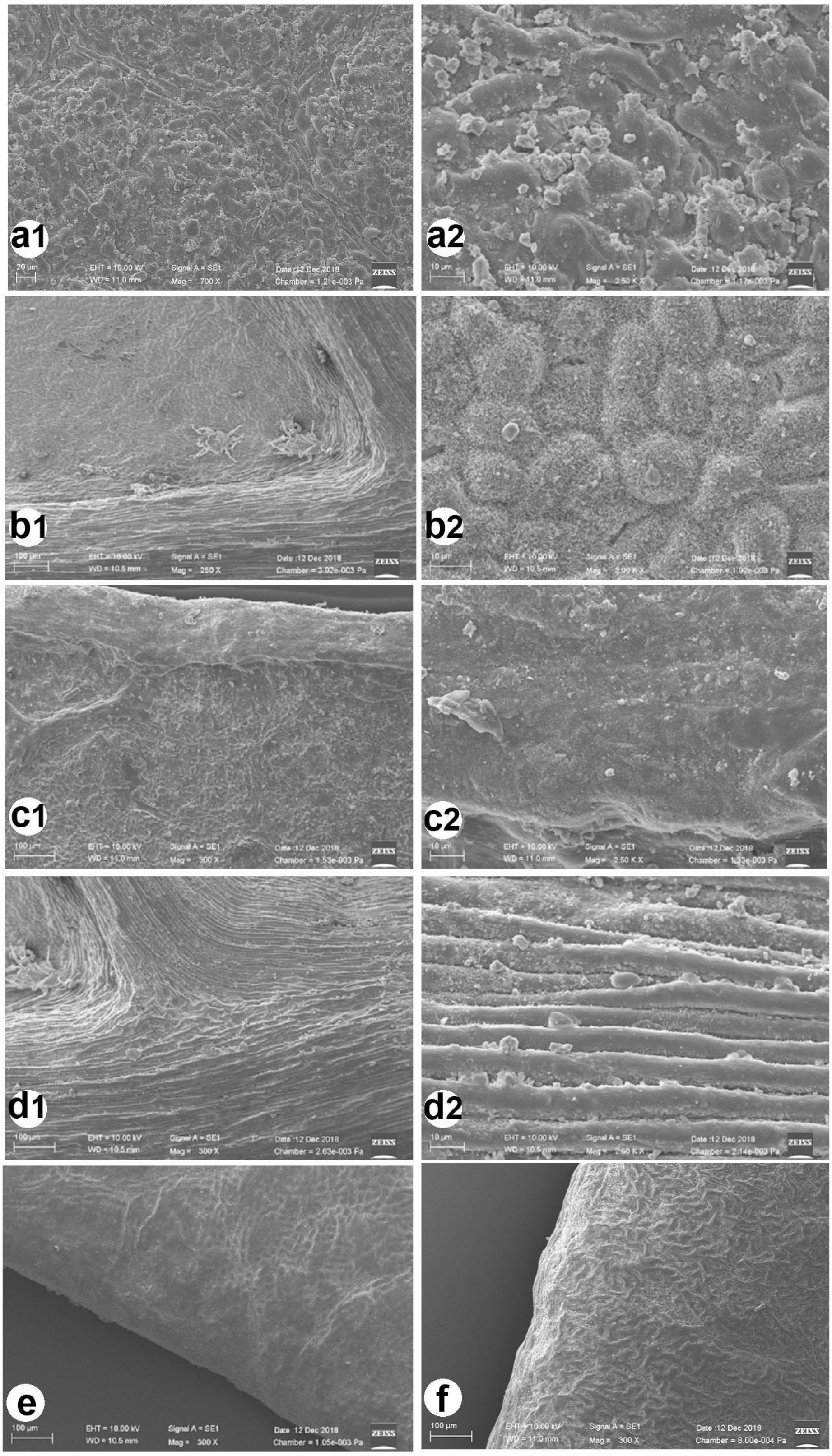

Figure 2. Leaf SEM micrographs of *C. coggygria*. a: upper surface with epicuticular wax, b: lower surface, c: upper surface of midrib, d: lower surface of midrib, e: upper surface of margin, f: lower surface of margin. Magnifications; a_1: 700X, a_2, d_2: 2500X, b_1: 250X, b_2: 3000X, c_1, d_1, e, f: 300X, c_2: 1000X.

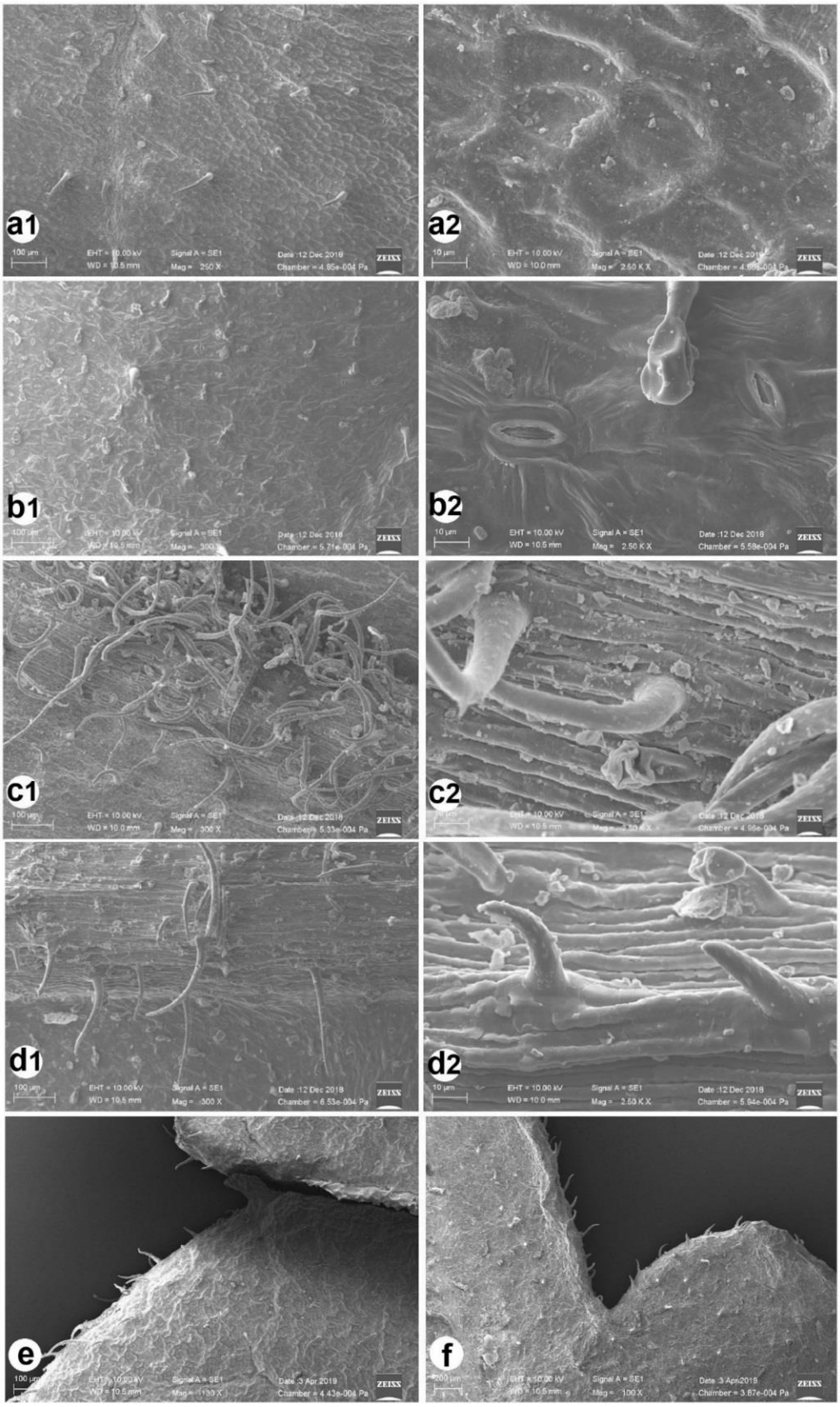

Figure 3. Leaf SEM micrographs of *R. coriaria*. a: upper surface, b: lower surface, c: upper surface of midrib with glandular and simple trichomes, d: lower surface of midrib, e: upper surface of margin with simple trichomes, f: lower surface of margin with simple trichomes. Magnifications: a_1, b_1: 250X, a-d $_{(2)}$: 2500X, c_1, d_1: 300X, e: 130X, f: 100X.

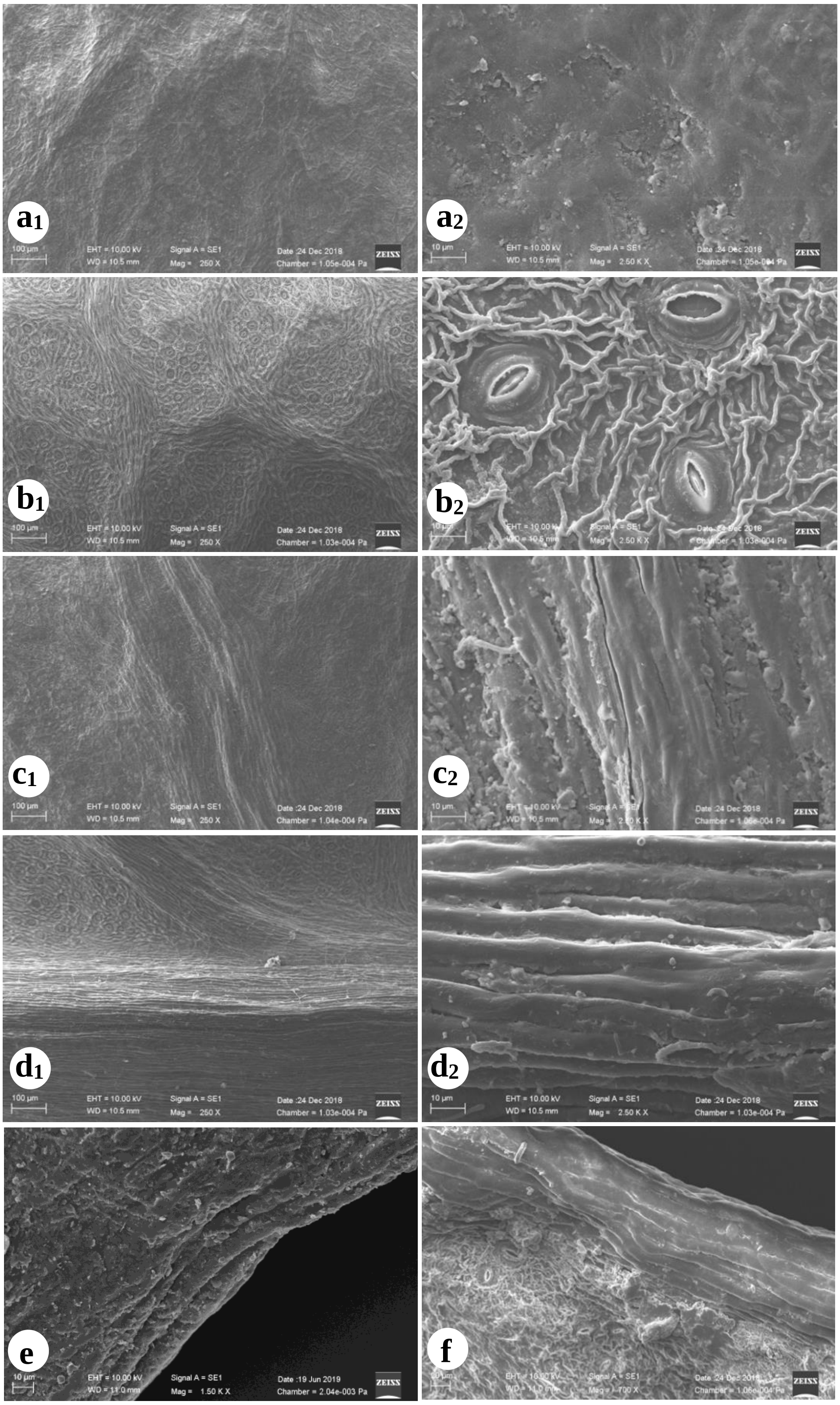

Figure 4. Leaf SEM micrographs of *Arbutus andrachne*. a: upper surface, b: lower surface with striate cuticle, c: upper surface of midrib, d: lower surface of midrib, e: upper surface of margin, f: lower surface of margin. Magnification; a-d ($_1$): 250X, a_2, b_2, d_2: 2500X, e: 1500X, f: 300X.

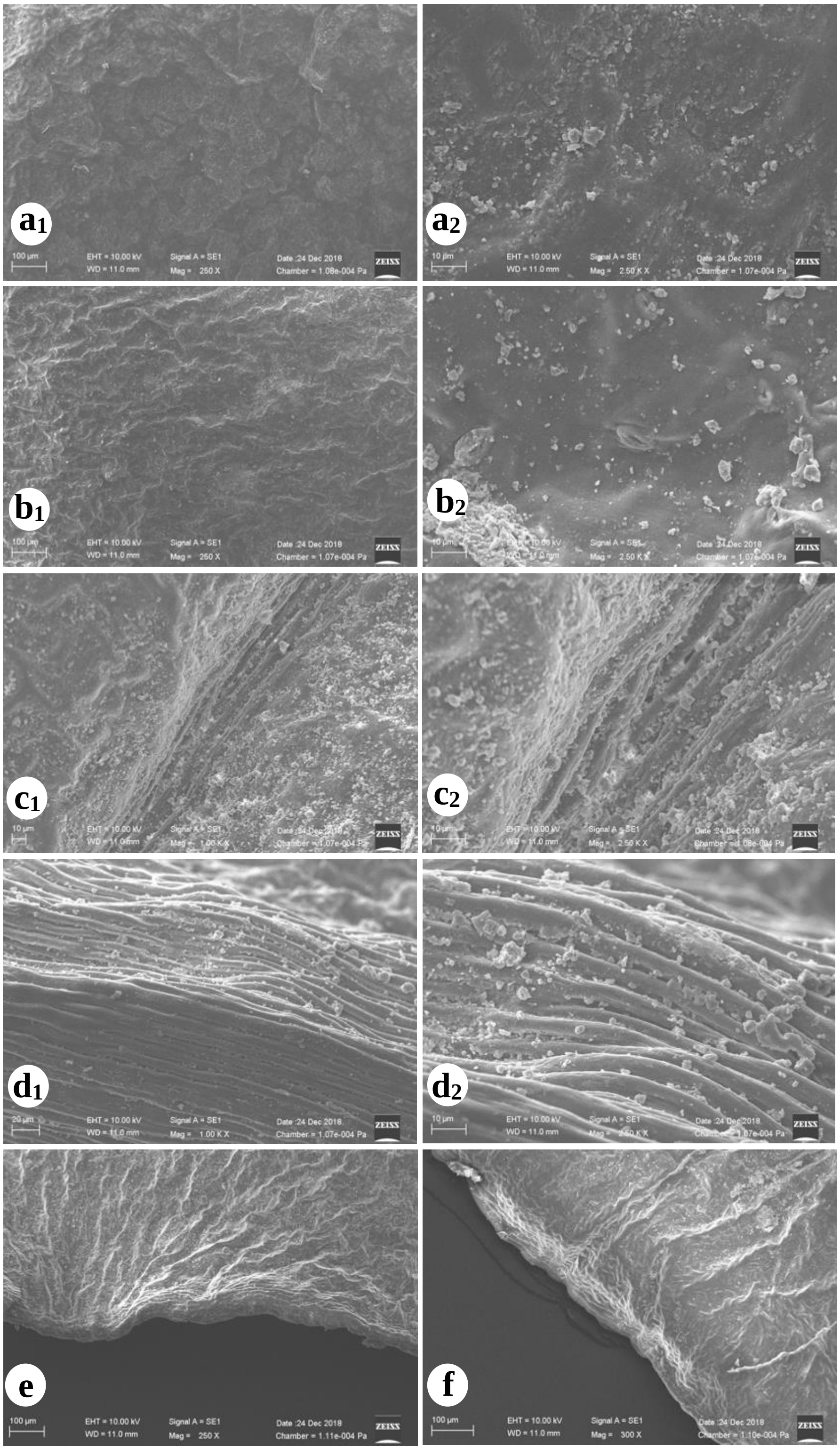

Figure 5. Leaf SEM micrographs of *P. granatum.* a: upper surface with epicuticular wax in the form of granules, b: lower surface with epicuticular wax in the form of granules, c: upper surface of midrib, d: lower surface of midrib, e: upper surface of margin, f: lower surface of margin. Magnification: $a_{1,}$ $b_{1,}$ e: 250X, a-d ($_2$): 2500X, $c_{1,}$ d_1: 1000X, f: 300X.

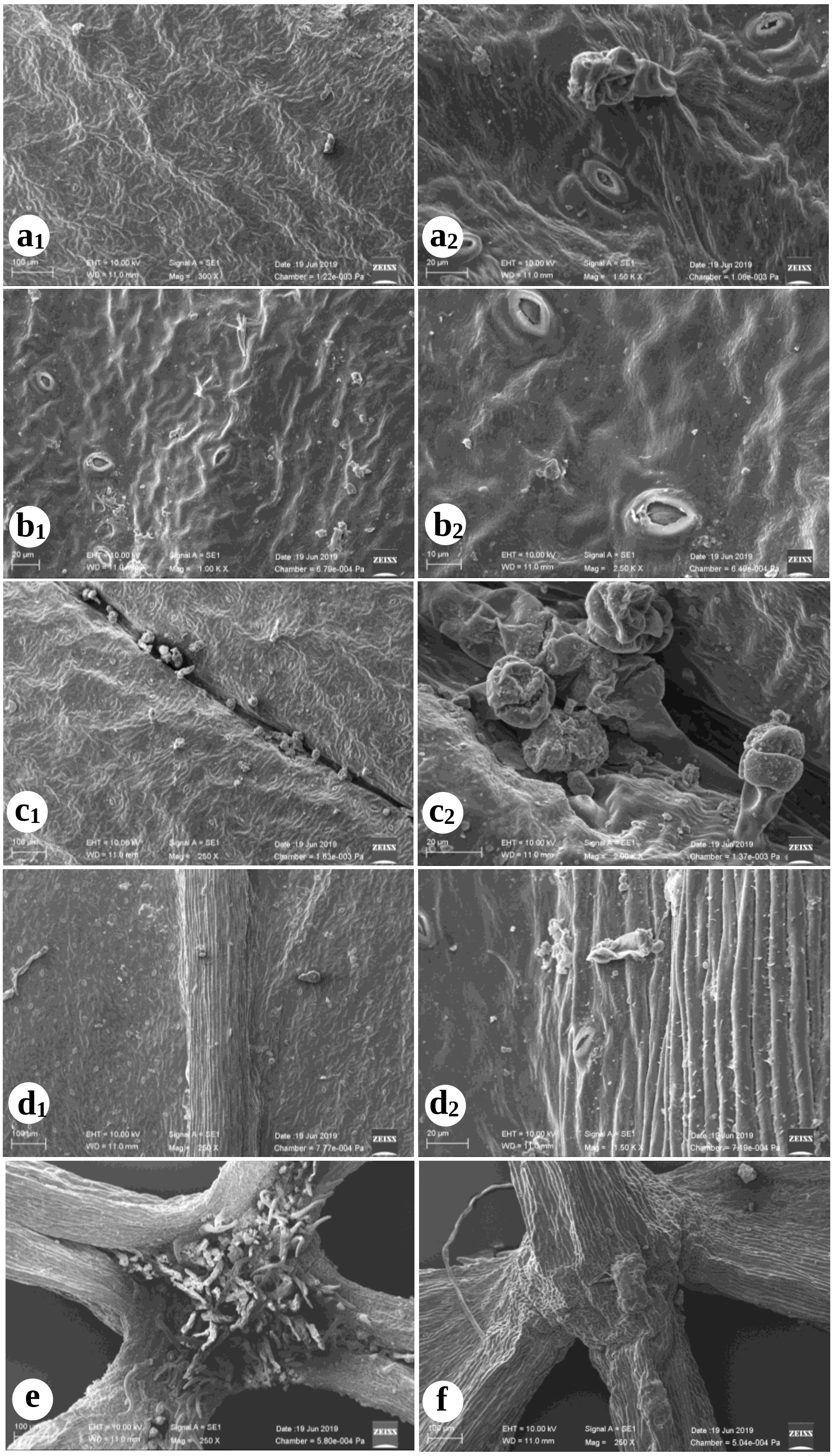

Figure 6. Leaf SEM micrographs of *J. fruticans*. a: upper surface, b: lower surface, c: upper surface of midrib with middle depression, d: lower surface of midrib, e: upper surface of central midrib with cluster of glandular and simple trichomes, f: lower surface of central midrib. Ölçek: a_1: 300X, a_2, d_2: 1500X, b_1: 1000X, b_2: 2500X, c_1, d_1, e, f: 250X, c_2: 2000X.

Anatomy
Petiole

In the two Anacardiaceae members, the petioles are circular, the other two species, however, have hemispherical to hemispherical-triangular shape structure and *J. fruticans* has horse-shoe shaped in outline. In *C. coggygria* and *R. coriaria*, round shape petioles are more or less angled and have parenchymatous pith in transverse sections. In *A. andrachne*, the petiole exhibited a flate side in upper part, while *P. granatum* and *J. fruticans* have shallow distinct depression in upper part. Epidermis is a single layer and rectangular shape in the species. No trichomes are observed in the epidermal surfaces of *C. coggygria* and *P. granatum*, whereas sparsely simple trichomes are shown in *A. andrachne*. Both eglandular and glandular trichomes are present in *R. coriaria* and *J. fruticans* and one layer-two layers of collenchyma in *C. coggygria* and six-nine layers in *R. coriaria* are almost surrounded the petiole. In the other three species, different layers of collenchyma are observed in the upper and lower surfaces of midrib region. The cortex is seven to ten layers and isodiametric shape in these two species, and seven to eight in *J. fruticans*. Plently of druses crystals are found in parenchymatous cells of cortex and phloem in *C. coggygria*, *R. coriaria* and *P. granatum*. Number of vascular bundles is four in *C. coggygria* and six to eight in *R. coriaria*, while the other two taxa have one large central bundle (Figures 7a, 8a). *A. andrachne* and *J. fruticans* have also two accessory bundle traces in the opposite directions. Vascular bundle is bicollateral in *P. granatum*, differently from others. Sclerenchymatous layers surround vascular bundles in *C. coggygria* and sclerenchymatous caps are present in the outsides of phloem parts of bundles of *R. coriaria*. On the other hand, small sclerenchyma cell clusters are shown in *A. andrachne* and *P. granatum*, but *J. fruticans* does not have these type cells in phloem parts. In the phloem parts of central vascular bundles and lateral veins, secretory canals are distinct with different numbers (8-10 in *R. coriaria*) and sizes, but only small secretory cells are visible in *J. fruticans*.

Leaf Blade

Shapes of leaf midrib are hemisperical in the two former species, hemisperical to triangular in the other three taxa. Adaxial surfaces are flat/convex in *C. coggygria* and more or less concave in *P. granatum.* One pronounced protrusion is visible in *R. coriaria*, while *J. fruticans* with a middle depression on the adaxial surface of midrib. Solitary and large vascular bundles are present in *A. andrachne* (arc-shaped) and *P. granatum* (arc-shaped), whereas two parts or two number of vascular bundles can be seen in the midrib of *C. coggygria*, *R. coriaria* and *J. fruticans* (Figures 7, 8 and 11). Schizogen secretory canals are present in the phloem parts of *C. coggygria* and *R. coriaria*. These canals are also visible in lateral veins of these two species. On the other hand, *J. fruticans* has many of small secretory cells in phloem part. They are in the phloem parts of *C. coggygria*, *A. andrachne* and sparsely in *P. granatum* with three layers and sometimes in small clusters, respectively. Near vascular bundles in the upper and lower directions, epidermises are sometimes three layers like hypodermal structure. Parenchyma cells near vascular bundles have druses crystals in *C. coggygria*, *R. coriaria* and *P. granatum*. Upper and lower surfaces of midrib of the investigated species have collenchyma cells with different layers. Collenchyma layers are distinct in the upper surfaces of *R. coriaria* and *A. andrachne*.

The cuticle is much thicker on the adaxial surface than on the abaxial surface, especially for *R. coriaria*, while the thinnest one occurs in *J. fruticans*. Epidermises comprise uniseriate cells. In terms of size, upper epidermal cells are much larger than those of the abaxial ones, except for *P. granatum*. Leaves are bifacial in the investigated species with one layer of palisade cells and different layers of spongy cells, but *A. andrachne* has three layers of palisade (Table 2). *C. coggygria*, *R. coriaria* and *P. granatum* have plently of druses crystals. *P. granatum* has also large single tetragonal crystals which they arise splitting the palisade cells.

Four species have hypostomatic type leaves, while *J. fruticans* has amphistomatic one. Anomocytic stomata are positioned on the lower surfaces at the behind of epidermal levels in *C. coggygria* and *A. andrachne*, whereas the stomata are found at the same level with neighboring cells of the other three taxa. Stomata are also surrounded with striate cuticle in *A. andrachne*. Waxy compound is densely visible in *C. coggygria* and *R. coriaria.* Average of stomatal frequencies ranged from 220 (*J. fruticans*) to 760 (*P. granatum*) per mm^2 in abaxial parts. In addition, *J. fruticans* has also 56 stomata per mm^2 in adaxial surfaces. Stomatal indices of abaxial surfaces vary from 22.74 in *P. granatum* to 9.69 in *A. andrachne*. On the other hand, stomatal indice on the adaxial surfaces of *J. fruticans* was found as 4.46. The largest stomata were identified on the adaxial surfaces of *J. fruticans*, while the smallest ones were found on the abaxial surfaces of *P. granatum*. Irregularly polygonal shape adaxial epidemal cells are found in the leaf surfaces of *J. fruticans*, while regulary polygonal epidermal cells are present in the remaining species examined. In the lower leaf surfaces of all species, anticlinal cell walls are determined as more or less sinuous shape.

Table 2. Petiole anatomical characteristics of investigated species

Character	*C. coggygria*	*R. coriaria*	*A. andrachne*	*P. granatum*	*J. fruticans*
Xylem thickness (µm)	156.40±3.28	190.81±19.88	264.44±4.86	96.70±5.20	163.53±17.3
Phloem thickness (µm)	180.56±13.46	149.42±11.92	119.20±6.00	inner: 99.92±2.39 outer: 54.90±7.86	71.69±6.32
Vascular bundle length (µm)	312.31±8.87	335.51±31.91	495.13±11.81	338.38±9.10	241.52±14.3
Vascular bundle width (µm)	416.02±8.42	322.90±22.56	800.35±5.25	618.31±15.74	405.91±20.6
Trachea diameter (µm)	21.81±1.45	25.26±1.96	16.05±0.27	13.17±0.91	14.52±1.06
Cortex thickness (µm)	46.52±3.27	57.16±11.05	82.96±5.72	55.81±3.96	118.81±7.35
Collenchyma thickness (µm)	66.43±6.60	50.71±6.35	321.87±10.57	288.83±15.35	46.94±22.90
Petiole diameter/width (µm)	993.49±25.92	1240.06±105.5	1237.02±10.9	1087.58±15.53	877.63±18.5

*mean±standar error; five samples were evaluated per species.

Table 3. Leaf anatomical features of investigated species

Character		*C. coggygria*	*R. coriaria*	*A. andrachne*	*P. granatum*	*J. fruticans*
Upper epidermis	length (µm)	28.11±10.52	23.03±0.91	16.45±0.85	20.10±0.85	17.06±0.48
	width (µm)	29.17±2.34	36.44±1.19	25.26±1.00	26.41±1.49	22.75±1.12
Lower epidermis	length (µm)	10.87±0.67	19.28±2.75	13.07±1.26	12.72±1.07	10.63±0.56
	width (µm)	32.87±12.85	25.05±1.36	24.42±1.78	18.54±1.45	15.86±0.97
Thickness of palisade layer/s (µm)		51.27±1.98	79.57±12.14	143.79±20.50	117.47±3.99	52.05±4.47
Thickness of spongy layers (µm)		58.14±6.77 (4-7 layers)	64.57±1.82 (3-4 layers)	111.01±8.21 (5-6 layers)	60.28±1.75 (4-5 layers)	91.82±4.51 (5 layers)
Midrib thickness (µm)		717.45±27.08	637.22±5.22	756.47±38.45	447.86±4.50	261.86±11.16
Lamina mesophyll thickness (µm)		143.92±12.64	116.32±15.21	312.04±16.03	219.76±5.80	143.10±5.01
Trachea diameter (µm)		23.21±0.97	22.52±0.52	16.38±0.65	10.14±0.23	9.22±0.35
Xylem thickness (µm)		239.32±14.41	85.27±6.18	148.27±4.71	68.88±4.16	49.18±4.11
Phloem thickness (µm)		162.60±15.15	118.74±16.26	118.20±3.24	inner: 50.33±9.50 outer: 36.83±2.17	52.06±5.75
Vascular bundle	Length (µm)	488.33±22.64	363.55±7.43	416.36±21.96	224.23±17.45	102.08±8.16
	Breath (µm)	427.92±13.61	518.66±20.74	620.91±21.96	276.98±22.77	142.49±20.06
Adaxial surface	Cuticle thickness(µm)	3.69±0.74	4.66±0.31	3.57±0.28	4.07±0.37	2.95±0.32
	Stomatal length (µm)	0	0	0	0	33.91±0.50
	Stomatal index	0	0	0	0	4.56±0.33
	Number of stomata per mm^2	0	0	0	0	56.0±11.64
Abaxial surface	Cuticle thickness (µm)	2.55±0.19	4.17±0.39	3.29±0.36	2.98±0.07	2.27±0.17
	Stomatal length (µm)	17.37±0.45	28.65±1.24	23.29±1.71	14.71±0.62	29.79±0.49
	Stomatal index	10.60±0.51	10.85±0.87	9.69±0.24	22.74±0.98	9.93±0.59
	Number of stomata per mm^2	448.0±34.34	298.0±36.32	224.0±29.88	760.0±35.71	220.0±19.96

*mean ±: standard error; five samples were evaluated per species.

Table 4. Main leaf characteristics of examined species

Species	Leaf type	Leaf morphology	Venation type	Crystal	Trichome types	Number of vascular bundle	Number of accessory bundles	Number of palisade layer	Presence of secretory canal/cell
Cotinus coggygria	Hypostomatic	Simple	Craspedodromous	Druses	Absent	Single and two parts (collateral) surrounded sclerenchymatous cap	-	1	Secretory canals (1-4)
Rhus coriaria	Hypostomatic	Imparipinnate	Craspedodromous	Druses	Simple and capitate glandular	Single and two parts (collateral)	-	1	Secretory canals (3-4)
Arbutus andrachne	Hypostomatic	Simple	Camptodromous-cladodromous	Absent	Sparsely and simple eglandular	Single (collateral)-groups of sclerenchmatous cell	2	3	Absent
Punica granatum	Hypostomatic	Simple	Camptodromous-brochidodromous	Druses and single tetragonal	Absent	Single (bicollateral)	-	1	Absent
Jasminum fruticans	Amphistomatic	Imparipinnate	Hyphodromous	Absent	Simple, short eglandular, and capitate and clavate glandular	Single (collateral)	2	1	Secretory cells (several)

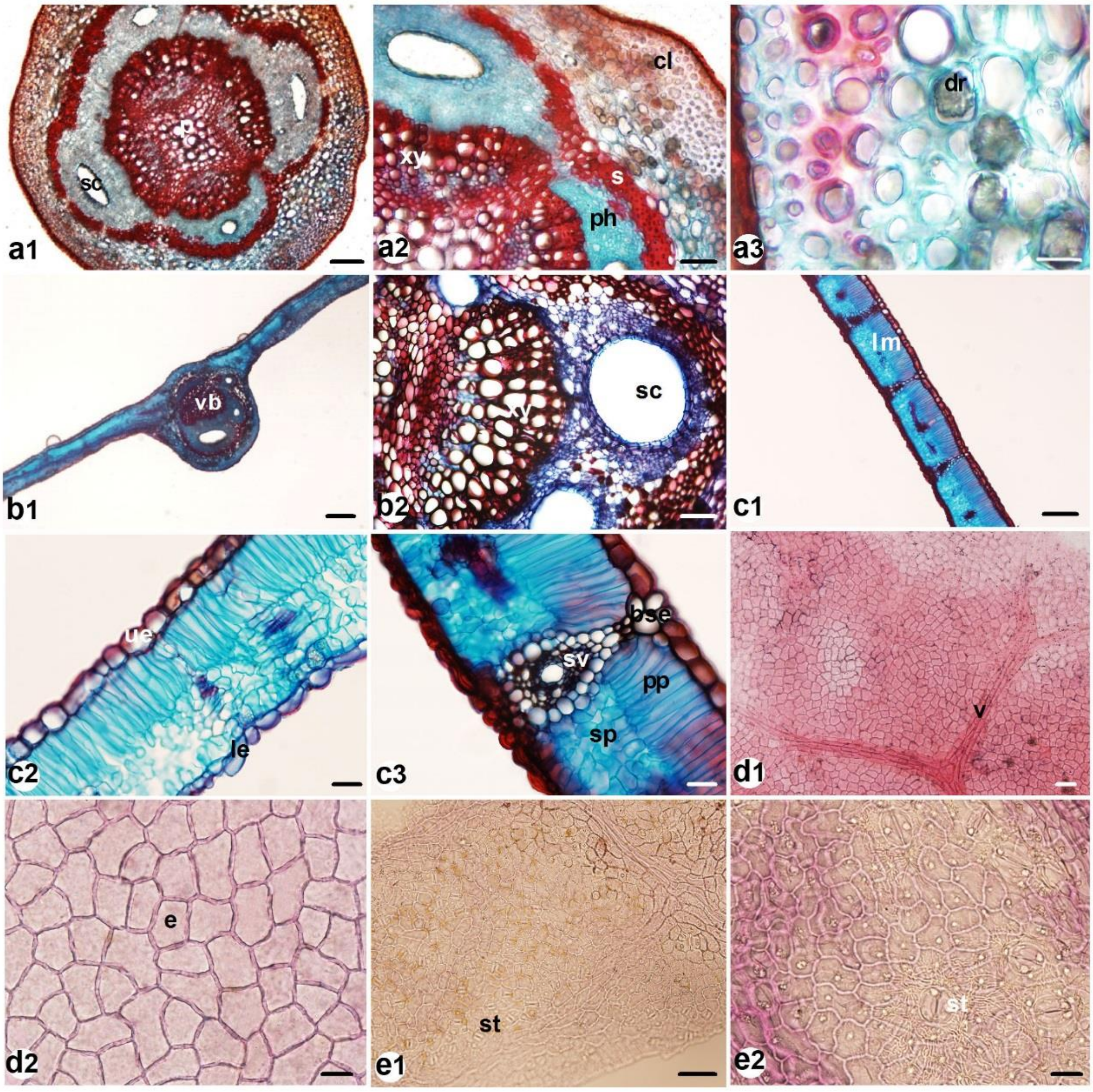

Figure 7. Leaf LM photomicrographs of *Cotinus coggygria*. a-c: transverse sections, d-e: paradermal sections. a: petiole, b: midrib, c: lamina. d: upper surface, e: lower surface. cl: collenchyma, dr: druses crystals, e: epidermis, le: lower epidermis, lm: lamina mesophyll, p: pith, ph: phloem, pp: palisade parenchyma, sc: secretory canal, sp: spongy parenchyma, st: stomata, sv: secondary vein, ue: upper epidermis, vb: vascular bundles, xy: xylem. Scale bars: 200 µm (a_1, b_1), 100 µm (a_2, c_1), 50 µm (b_2, d_1, e_1), 20 µm (a_3, c_2, c_3, d_2, e_2).

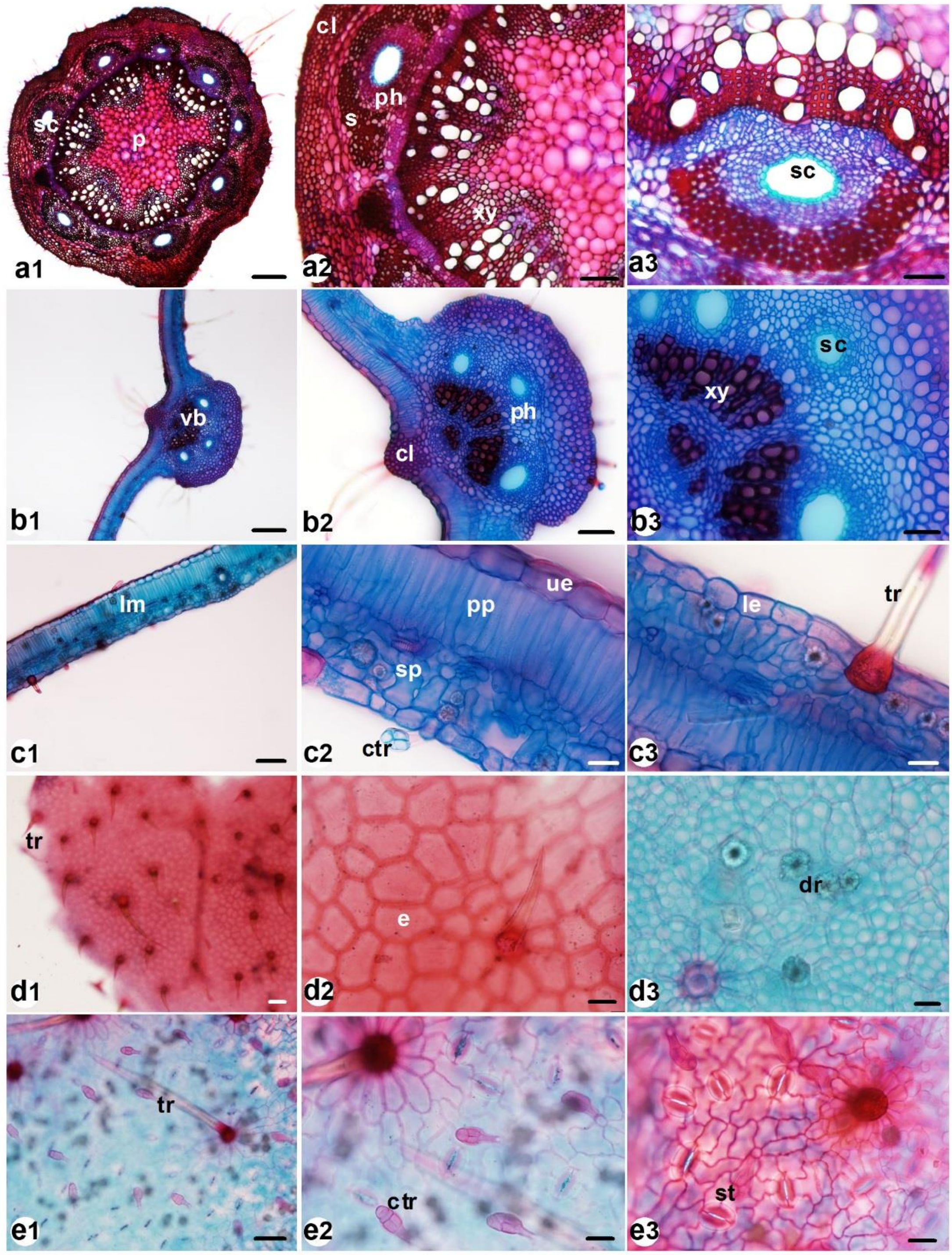

Figure 8. Leaf LM photomicrographs of *R. coriaria*. a-c: transverse section, d-e: paradermal section. a-c: petiole, b: midrib, c: lamina. d: upper surface, e: lower surface. ctr: capitate glandular trichome, tr: simple trichome. See Figure 7 for other abbreviations. Scale bars: 200 µm (a_1, b_1), 100 µm (a_2, c_1, d_1), 50 µm (a_3, b_2, e_1), 20 µm (b_3, c_2-c_3, d_2-d_3, e_2-e_3).

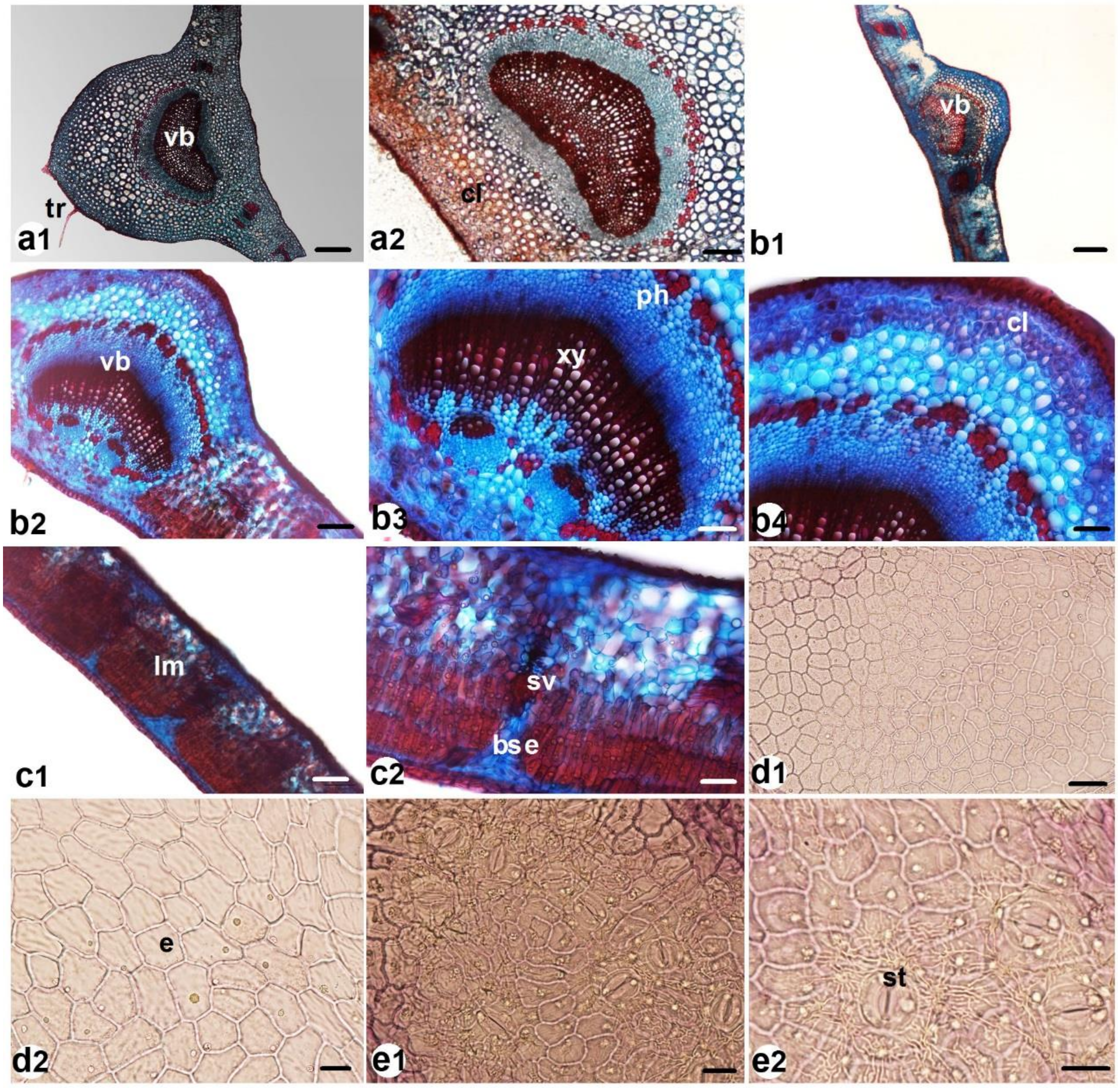

Figure 9. Leaf LM photomicrographs of *Arbutus andrachne*. a-c: transverse sections, d-e: paradermal sections. a: petiole, b: midrib, c: lamina. d: upper surface, e: lower surface. bse: bundle sheat extention. See Figure 7 for other abbreviations. Scale bars: 200 µm (a_1, b_1), 100 µm (a_2, b_2, c_1), 50 µm (b_3-b_4, c_2, d_1, e_1), 20 µm (d_2, e_2).

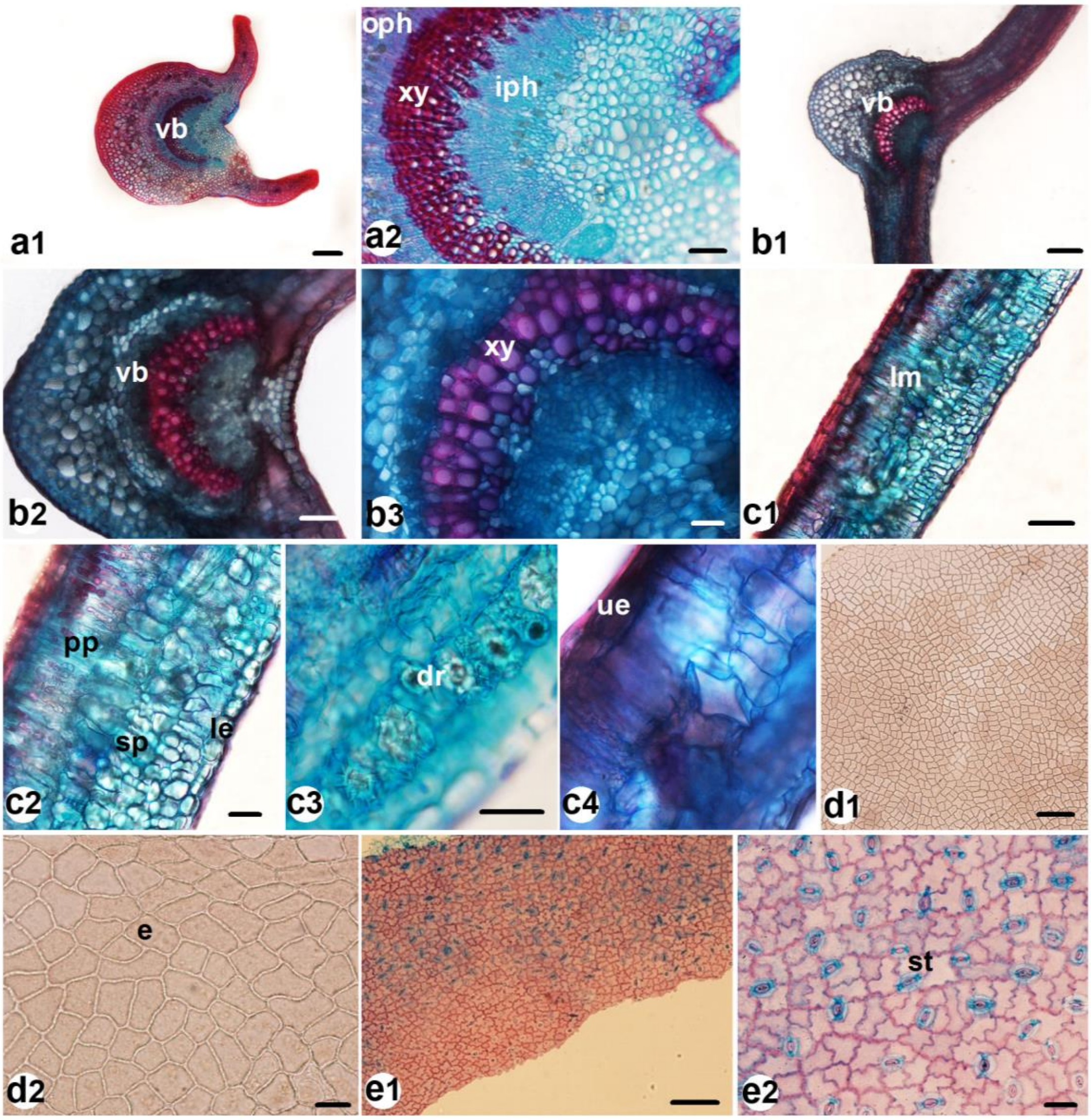

Figure 10. Leaf LM photomicrographs of *P. granatum*. a-c: transverse sections, d-e: paradermal sections. a: petiole, b: midrib, c: lamina. d: upper surface, e: lower surface. See Figure 7 for abbreviations. Scale bars: 200 μm (a_1), 100 μm (b_1, c_1), 50 μm (a_2, b_2, d_1, e_1), 20 μm (b_3, c_3-c_4, d_2, e_2).

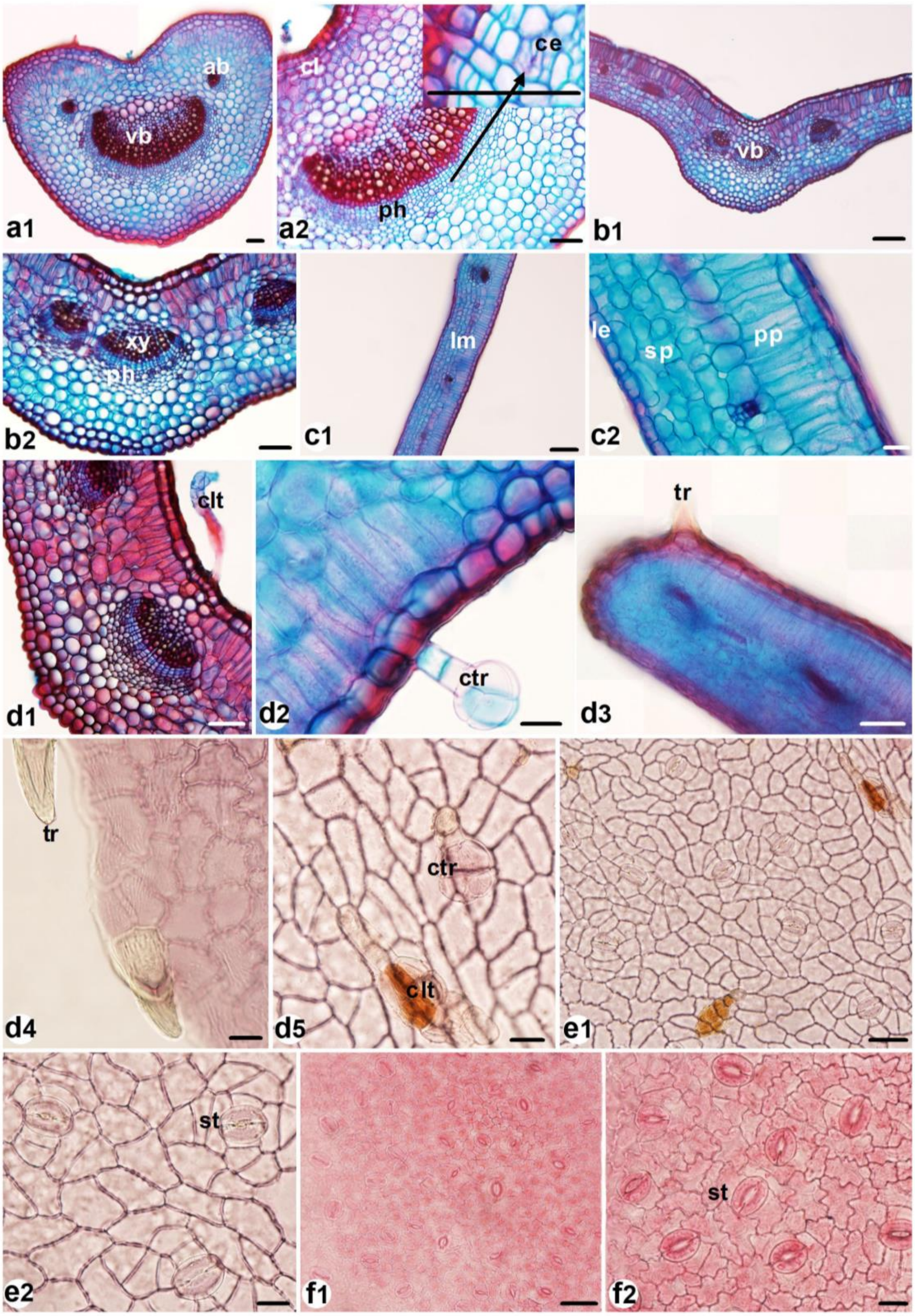

Figure 11. Leaf LM photomicrographs of *J. fruticans*. a-c, d_1-d_3: transverse sections, d_4-d_5, e-f: paradermal sections. a: petiole, b: midrib, c: lamina. d: trichome types (1-clt: clavate trichome in midrib, 2-ctr: capitate trichome with multicellular head cells in midrib, 3,4-tr: simple trichomes in leaf margins, 5-ctr, clt: clavate and capitate trichomes in upper surfaces), e: upper surface, f: lower surface. See Figure 7 for abbreviations. Scale bars: 100 mm (a_1, b_1, c_1), 50 mm (a_2, b_2, c_2, d_1, d_3, e_1, f_1), 20 mm (d_2, d_4, d_5, e_2, f_2).

Discussion

The morpho-anatomical characteristics of leaves of five Mediterranean enclaves were examined in our investigation. They are also medicial plants using different aspects such as decoction, sprey and anti-inflammatory (Nagarajan, 1982; Kuwajima et al., 1985; Tewari et al., 2001; Demirci et al., 2003; Tanker, et al., 2007; Matić et al., 2011; Prakash & Prakash, 2011; Gospodinova et al., 2017), and cultivated for their fragrance of flowers (Tewari et al., 2001; Kaviarasan et al., 2015).

The two of examined species; *Rhus coriaria* and *Jasminum fruticans* have compound leaves, whereas simple leaves are present in the other ones. Imparipinnately compound leaf character is shared between *R. coriaria* and *J. fruticans* as mentioned in literature (Davis, 1967; Chamberlain, 1972; Yaltırık, 1967). Leaves are deciduous in the species, except for *A. andrachne*. Similarly, other Mediterranean species; *Laurus nobilis* L. and *Olea europaea* L. are evergreen, but *Cistus* species are not.

Leaf/leaflets margins of *C. coggygria*, *P. granatum*, *J. fruticans* are simple, however, distinct and slightly serrate leaf/leaflet margins are observed in *R. coriaria* and *A. andrachne*, respectively. *C. coggygria*, *P. granatum* and *J. fruticans* have truncate leaf tips, but *R. coriaria* and *A. andrachne* have acute ones. Morphological findings are in agreement with previous reports described by Davis (1967), Chamberlain (1972) and Yaltırık (1967).

In terms of venation types, craspododromous venation is found in the leaves of *C. coggygria* and *R. coriaria*, camptodromous-brochidodromous in the leaves of *P. granatum* and camptodromous-cladodromous one in the leaves of *A. andrachne* (Figure 1). Venation types of *C. coggygria*, *A. andrachne* and *J. fruticans* are given here for the first time. Andréz-Hernández & Terrazas (2009) described craspedodromous, eucamptodromous and cladodromous venations in the genus *Rhus* s.str., and craspedodromous and eucamptodrous venations were reported for the subgenus *Rhus*. In addition, these authors mentioned that craspedodromous venation has been observed in the species which have compound and deciduous leaves. This idea is true for *R. coriaria* and our findings are in agreement with Andréz-Hernández & Terrazas (2009) report. From one report (Panda & Chowdhury, 2010) about venation type of *Rhododendron vaccinioides* belonging to the family Ericaceae, it has been observed that secondary and tertiary veins are joined without terminating at the margins (camptodromous). In *A. andrachne,* however, secondary and tertiary veins are not reach to leaf margins, but gradually iminished/ramified (eucamptodromous-cladodromous type venation). Hickey (1973) and Lersten & Horner (2005) previously reported brochidodromous venation type for *P. granatum* as in our examinations. For *J. fruticans*, we determined hyphodromous venation in which only the primary vein is visible, but secondary and tertiary veins are not, because of coriaceous leaf/leaflet character.

Leaves of *C. coggygria, A. andrachne* and *P. granatum* have no trichomes, but long and sparsely simple trichomes are observed in the petiole of *A. andrachne*. On the other hand, *R. coriaria* and *J. fruticans* have both eglandular and glandular trichomes. These glandular trichomes are densely and capitate in *R. coriaria*, while both capitate and clavate ones are present in *J. fruticans*. Simple trichomes and capitate glandular trichomes have been also previously reported by Eminağaoğlu & Ozcan (2018) in *R. chinensis* Mill. Ali & Sosa (2015) studied epidermal characters of four *Jasminum* species from gardens throughout Iraq and reported eglandular trichomes for *J. grandiflorum* L. and *J. officinale* L., and both eglandular and glandular ones for *J. sambac* (L.) Aiton and *J. mesnyi*. Solereder (1908) and Metcalfe & Chalk (1950) also reported three trichome types in the family Oleaceae. In addition, Inamdar (1967) examined different species in Oleaceae family which four of them belonging to the genus *Jasminum* (*J. auriculatum* L., *J. flea* Vahl, *J. officinale* L., *J. sambac* Ait.) and described 12 different trichome types that the capitate filiform type was not determined in *Jasminum* species.

Striate cuticular structure in the abaxial surface of *A. andrachne* is evident, whereas the remaining taxa investigated have more or less smooth surface. Bačić et al. (1992) has previously reported striate structure for *A. andrachne*. Anticlinal walls of *C. coggygria* are sunken and periclinal walls are convex, while distinct anticlinal walls and sunken periclinal cell walls are present in *R. coriaria*. In *A. andrachne*, adaxial surface is undulate, anticlinal cell walls are not distinct and periclinal ones are ruminate. Upper leaf surface of *P. granatum* is undulate, anticlinal walls are ruminate, while priclinal ones are sunken.

Cross sections of petioles are circle (*C. coggygria* and *R. coriaria*), triangular-hemispherical (*A. andrachne*) and hemisperical (*P. granatum*) in investigated species. As arranged one circle, four vascular bundles in *C. coggygria*, six to eight with different sizes in *R. coriaria* were determined, while *A. andrachne*, *P. granatum* and *J. fruticans* have only one vascular bundle. *A. andrachne* and *J. fruticans* have also two accesssory bundle traces. Main bundles are found as closed collateral in examined species excluding *P. granatum* that has bicollateral vascular bundle, differently from the others. Rajaei & Yazdanpanah (2015) and Metcafe & Chalk (1950) have also previously described bicollateral bundle for this species. Esau (1997) and Fahn (1990) mentioned that inner phloem in leaves are not usual event. In addition to *P. granatum*, Yentür (2003) reported this type vascular bundle for the genus *Nerium*. *C. coggygria* and *R. coriaria* possess large secretory canals in their phloem parts of vascular bundles. In one previous report, Antal et al. (2015) mentioned these structures in *C. coggygria.* Furthermore, Eminağaoğlu & Ozcan (2018) reported them for *R. chinensis.*

Three of examined species have crystals in parenchymatous cells with different type and density, but *A. andrachne* and *J. fruticans* are without crystals. However, Bačić et al. (1992) reported them in the bundle sheats of *A. andrachne*. Ali & Sosa (2015) reported tetragonal or druses crystals only in *J. sambac* among their studied *Jasminum* species. We found druses crystals in *R. coriaria*. Similarly, Eminağaoğlu & Ozcan (2018) mentioned them for *R. chinensis.* In addition to druses crystals, *P. granatum* has also single tetragonal crystals in their leaf lamina. Metcalfe & Chalk (1950) and Lersten & Horner (2005) previously reported both prismatic and druses crystals for *P. granatum*. Druses crystals in parenchymatous tissue of sepals for *P. granatum* was determined by Meera Devi Sir et al. (2015).

All species examined have dorsiventral mesophyll (bifacial leaf). On the other hand, number and thickness of palisade and spongy parenchyma differ among the species, and thus represent diagnostic anatomical characters. Four species have a single layer of parenchyma, but *A. andrachne* has three palisade layers, differently from the others. Fahn & Cutler (1992) reported that an increase in the amount of palisade parenchyma is related to high light intensities of plants. Also, palisade parenchyma thickness of *P. granatum* is bigger than spongy parenchyma thickness. On the other hand, palisade parenchyma cells structure is very similar to spongy parenchyma in the species. Rajaei & Yazdanpanah (2015) and Fahn (1990) reported bifacial leaf for *P. granatum* and noted that it is difficult to exactly distinguish the palisade layer from spongy layers. In *A. andrachne*, three palisade layers in leaf lamina are interrapted by bundle sheat extentions, as in the leaf blade of *C. coggygria*. Bačić et al. (1992) reported from Yugoslavia that these extentions are similar to hypodermal cells. Antal et al. (2015) also previously mentioned from these cells in the leaf of *C. coggygria.* Among the investigated taxa, the thickest palisade layer was measured in *A. andrachne* (143.79 µm), while the thinnest one is observed in *C. coggygria* (77.79 µm). *A. andrachne* has also the thickest leaf lamina mesophyll (Table 3). In the midrib of leaves, one bundle occurs in *A. andrachne* and *P. granatum*, one bundle with two parts in *C. coggygria* and *R. coriaria*, and one-two vascular bundles in *J. fruticans*. *C. coggygria* and *R. coriaria* belonging to the family Anacardiaceae have secretory canals with in different numbers in the laminae and petioles. Our findings are in agreement with literature (Antal et al., 2015; Eminağaoğlu & Ozcan, 2018). In the median portion of leaf of *R. coriaria*, one distinct protrusion was also detected in adaxial leaf surface.

Among the examined species, *J. fruticans* only has amphistomatic leaf, differently from the others, but all species have anomocytic type stomata. This type of stoma has been previously reported by Antal et al. (2015) for *C. coggygria*, by Metcalfe & Chalk (1967) for *P. granatum*, and by Eminağaoğlu & Ozcan (2018) for *R. chinensis*. Metcalfe & Chalk (1950) also reported anomocytic type stomata in the family Oleaceae. Wilkinson (1979), however, reported cyclocytic or stephanocytic type stomata in the abaxial surface of *A. andrachne*, differently from our study.

Ali & Sosa (2015) studied four *Jasminum* species from Iraq and determined anomocytic stomata for them. They reported only hypostomatic leaves in their studies species, different from *J. fruticans* examined in this study. Hypostomatic leaves or reduced leaf area in most species can be interpreted as morphoanatomic adaptation to water deficiency and drought conditions. Similar adaptations were reported in the anatomy of the pampas biome (family Asteraceae) by Liesenfeld et al. (2019). In terms of the numbers and indices of stomata per mm^2, *P. granatum* has the biggest values (760 and 22.74), while *A. andrachne* has the lowest ones (224 and 9.69). *A. andrachne* grows well in forest habitat, but *P. granatum* present in roadsides or sunny slopes in where the influence of temperature is higher and water availability is lower. Narrower/reduced, deciduous and hypostomatic leaf characters of *P. granatum*, and the occurence of epicuticular wax are an adaptation to arid (xeromorphic) habitats that water loss/deficiency take places. Striate cuticular ornamentation in *A. andrachne* also provides protection against excessive evaporation from large leaves and decrease the influence of high light intensity to the leaf structure. Pătrut et al. (2005) previously mentioned from this feature in literature. Similarly, the ribbed cuticular thickening has been reported for *Xeranthemum annuum* and *X. cylindraceum* by Gavrilović et al. (2018). In addition, the coriaceous leaf characteristic of *A. andrachne* supplies stronger leaves and adaptive strategy as previously reported in some Asteraceae species belonging to the Pambas biome (Liesenfeld et al., 2019). It has been mentioned that coriaceous leaves are related with evergreen species (Turner, 1994).

The leaf micromorphological and anatomical characters obtained in the present study seems to be informative at a species level and explain their adaptation strategies to their growing habitats and contribute their knowledges. On the other hand, to compare ecological regions or climates exactly, measurements, calculations and interpretations on the same species from Euro-siberian and Mediterranen phloristic regions can be evaluated together.

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