



Morphological and anatomical studies on a rare species *Rhamnus depressa* Grub. (Rhamnaceae): its differences from *R. microcarpa* Boiss.

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Abstract

In this study, morphologic-micromorphologic, wood, and leaf characteristics of *R. depressa*, a rare species and known only from the type locality, were described for the first time and evaluated comparatively with *R. microcarpa*. The morphological and anatomical characteristics such as petiole length and leaf sizes, and numbers of bud scale, seed, and leaf veins, ray characteristics in wood, amphistomatic stomata, horseshoes vascular bundle in the petiole, one vascular bundle or two vascular bundles with sclerenchymatous cells in the phloem part, as well as micromorphological characteristics can be used in differentiating *R. depressa* and *R. microcarpa*. Especially, stomatal characteristic (amphistomatic) is significantly important and can be used to differentiate these two closely related species.

Key words: *Rhamnus depressa*, anatomy, micromorphology, Artvin, Turkey

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Nadir tür *Rhamnus depressa* (Rhamnaceae) üzerine morfolojik ve anatomik çalışmalar: *R. microcarpa*'dan farklılıkları

Özet

Bu çalışmada, nadir bir tür olan ve sadece tip lokalitesinden bilinen *R. depressa*'nın morfolojik ve mikromorfolojik karakterleri, odun ve yaprak özellikleri ilk kez tanımlanmış ve *R. microcarpa* ile karşılaştırmalı olarak değerlendirilmiştir. Yaprak sapı uzunluğu ve yaprak boyutları, tomurcuk pulları, tohum ve yaprak damarlarının sayıları, odunda özışını özellikleri, amfistomatik stomalar, yaprak sapında at nalı şeklindeki vasküler demet, floemde sklerenkima hücreleri ile çevrili bir veya iki iletim demeti gibi morfolojik ve anatomik özellikler, mikromorfolojik özelliklerinin yanı sıra, *R. depressa* ve *R. microcarpa*'yı ayırt etmede kullanılabilir. Özellikle, stoma özelliği (amfistomatik) yakın ilişkili bu iki türü ayırt etmede oldukça önemlidir ve ayırım için kullanılabilir.

Anahtar kelimeler: *Rhamnus depressa*, anatomi, mikromorfoloji, Artvin, Türkiye

1. Introduction

The plant family Rhamnaceae Juss. is cosmopolitan in distribution mostly found in tropics and warm temperate regions and comprises of 11 tribes, 62 genera and 1184 species [1-3]. This family has 6 genera including *Atadinus* Raf., *Rhamnus* L., *Paliurus* Mill., *Ziziphus* Mill., *Sageretia* Brongn., *Frangula* Mill. Hauenschild et al. [4] evaluated a total of five species as *Atadinus* genus, including *R. microcarpa* and *R. depressa*, however consideration of this subject continues to be discussed. The genus *Rhamnus* is represented by 22 taxa distributed different parts of Anatolia belonging to three sections (*Espinosa* DC., *Alaternus* DC. and *Rhamnus* L.) and six of them are endemic in Turkey.

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Five *Rhamnus* species including *R. imeretinus* Booth, Petz. & Kirchn., *R. microcarpa* Boiss., *R. depressa* Grub., *R. catharticus* L. and *R. pallasii* Fisch. & C.A.Mey. have been recorded in NE Anatolia. Three out of five taxa are shrubs up to 2-6 m., while *R. microcarpa* and *R. depressa* are known low shrubs. Both of the two species have similar distribution of the World; Northern Caucasus, Georgia [Caucasus], Armenia, NE-Turkey. *R. depressa* spreads in a local area than *R. microcarpa* in Turkey. While *R. depressa* grows only in Artvin and Kars, *R. microcarpa* shows a wider distribution (Giresun, Gümüşhane, Erzincan, Rize, Artvin) [5-7].

Besides the morphological characteristics, taxonomic importance of anatomical and micromorphological features are also given in several studies [8, 9]. There are few micromorphological and anatomical studies on *Rhamnus* species in the world. In earlier studies, it was determined the wood anatomy characteristics of some *Rhamnus* species in Turkey and the world [10-17]. In the family Rhamnaceae, anatomical characteristics of some genera (i.e *Rhamnus* L., *Retanilla* (DC.) Brongn.) and also some taxa (*Frangula alnus* Mill. subsp. *pontica* (Boiss.) P.H.Davis & Yalt.) have been previously reported by the authors [17-22].

Rhamnus depressa is only known from type locality and evaluated in 'DD' (Data Deficient) category [5]. This species was collected by Eminağaoğlu et al. [7]. It has been mentioned in literature that specific distinctness of *R. depressa* from *R. microcarpa* needs confirmation [5]. It has minor morphological differences between *R. depressa* and *R. microcarpa*, and no detailed study was performed on the micromorphology and anatomy of *R. depressa*. In this context, the present study is aimed to characterize and document the wood and leaf anatomical, and micromorphological features of this species for the first time and also to distinguish it more precisely from closely related species; *R. microcarpa*.

2. Materials and methods

2.1. Morphological examination

Plant specimens were collected between the years 2016 and 2017 in Şavşat (Artvin), photographed, and then GPS coordinates were taken. According to herbarium methods, all samples were dried. Plant identification was completed by the keys given in Flora of Turkey [23, 24]. Revision of important taxonomic characters was carried out and measurements of them were performed. Plant samples were stored at the Herbarium of Artvin Çoruh University (ARTH), Artvin, Turkey. The plant name was checked using International Plant Names Index [25], The Plant List [26], Catalogue of Life [3] and Türkiye Bitkileri Listesi-Damarlı Bitkiler [27].

2.2. Anatomical preparation

Thin wood sections in three directions, transversal, tangential and radial, were taken by using sliding microtome. Sections stained with a safranin O and alcian blue combination [28]. Macerations were prepared using Schultze's method [29]. All wood terms were determined according to the International Association of Wood Anatomists Committee on Nomenclature [30].

Fresh specimens used for this study were fixed in FAA (Formal 5 ml + glacial acetic acid 5 ml + %70'lik ethyl alcohol 90 ml) and stored in 70% alcohol for leaf anatomical studies. Transverse sections were taken from the middle part of mature leaf, and paradermal sections of upper and lower epidermis of leaves were prepared manually using commercial razor blades and stained in Haematoxylin for about 15 min. To remove the excess stain, sections were washed in water several times [31]. Semi-permanent slides were mounted in glycerin and permanent slides were covered with glycerin-gelatin [32].

Sections were examined under a light microscope and photographs were taken by using an Olympus BX-53 microscope with digital camera attachment DP-73. For all characteristics, mean values were based on 30 measurements or counts.

2.3. Micromorphological studies

Leaf micromorphological features of *R. depressa* were characterized using a stereomicroscope and a scanning electron microscope. The leaves were first examined using a stereomicroscope to determine shape, color and maturity (Leica M60 with a digital camera attachment DFC 295). For scanning electron microscopy (Zeiss Evo LS 10), dried and mature upper and lower leaf parts were separately placed on stubs using double-sided adhesive tape, and coated with gold in a Cressington sputter coater 108 auto coating apparatus for 2 minutes. They were examined and photographed from the same region (from the middle part and margin of the leaf).

3. Results

In this investigation, wood and leaf characteristics of *R. depressa* were described and evaluated comparatively with *R. microcarpa*. *R. depressa* has been known only from type locality, till now [5, 7]. In literature, its morphological description is not sufficient and detailed survey is necessary for this species. Therefore, it could not be exactly discriminated from *R. microcarpa* morphological aspects. *R. depressa* was collected again from the type locality and, detailed morphological features, micromorphological and anatomical properties of its were given for the first time.

3.1. Morphology

Rhamnus depressa Grubov in Not. Syst. Herb. Inst. Acad. Sci. URSS 12: 126 (1950).

Syn: *R. microcarpa* Boiss. var. *microphylla* Trautv. in Acta Horti Petrop. 4: 123(1867); *R. microcarpa* var. *acutifolia* Medw. in Mon. Jard. Bot. Tiflis 25:3(1912); *Oreohertzogia depressa* (Grub.) Vent in Feddes Rep. 65: 101 (1962)! Ic:Fl. Gruzii 6: t. 260(1950).

Low shrub and deciduous, usually much branched. Branches opposite or alternate, unarmed, glabrous; winter buds with scales. Young twigs puberulent or glabrous, bud scales 2, glabrous, 1.5-2mm.

Leaves alternate, broadly ovate or oval, 0.8-3.2 x 0.4-1.8 cm, acute or obtuse not fasciculate; stipules 2-5.5 mm, mainly subulate, caducous, rarely persistent; leaf blade always undivided, pinnately 5-7 veined, margin crenate-serrate, upcurved, glabrous on both surfaces, dark green above, yellowish green below, usually attenuate, cuneate or unequal at base, petiole 2-6.5 mm, puberulent.

Inflorescence of solitary or few fascicled in axillary cymes, flowers 2.5-3 mm, mostly yellowish green, small, bisexual or unisexual, rarely polygamous, pedicel 3.5-4.5 mm, puberulent. Calyx tube campanulate to cup-shaped; sepals 4 or 5, ovate-triangular, adaxially ± distinctly keeled. Petals 4, rarely absent, shorter than sepals, cucullate to hooded, often enfolding stamens, base shortly clawed, apex often 2-fid. Stamens 4, surrounded by and shorter than petals; anthers dorsifixed. Disk thin, adnate and lining calyx tube. Ovary superior, globose, free, 2-4-loculed; styles ± deeply 2-4-cleft. Fruit 3-4 x 2.5-3.5 mm, red-berrylike drupe, obovoid-globose or globose; seeds 3-4 x 1.5-2 mm, obovoid or oblong-obovoid, unfurrowed or abaxially or laterally with a ± long, narrow to gaping, often distinctly margined furrow; endosperm fleshy. Seed 7-8. On dry slopes and adpressed to rocks, 1200-1300 m (Table 1; Figure 1).

Table 1. Some morphological characteristics of *R. depressa*, differing from *R. microcarpa*

Characteristics	<i>R. depressa</i>	<i>R. microcarpa</i> [17]
Bud length	1.5-2 mm	1-4 mm
Number of Bud scale	2	5-7
Petiole length	2-6.5 mm	6-15 mm
Leaf length	0.8-3.2 cm	3.0-5.5 cm
Leaf width	0.4-1.8 cm	1.9-3.9 cm
Flower length	2.5-3 mm	2.5-4 mm
Pedicel in flower	3.5-4.5 mm	2.5-7 mm
Stipule	2-5.5mm	4-7 mm
Fruit length	3-4 mm	3-5 mm
Fruit width	2.5-3.5 mm	3-5 mm
Pedicel in fruit	2-6.5 mm	2.5-7 mm
Number of seeds	7-8	3-4
Seed length	3-4 mm	3-4 mm
Seed width	1.5-2 mm	2-3 mm
Number of leaf veins	5-7	7-10

Flowering period: May; *fruiting period:* July.

Specimens examined: Turkey – Artvin, Şavşat, Meydancık, Dutlu to Akdamla, rocky slope, 1221 m, 41° 23' 17" 42° 21' 00", 23.08.2016; *Ö.Emin.* 22363 (ARTH 11803); 1165 m, 41° 23' 08" 42° 20' 50", 08.05.2017, 10.07.2017; *Ö.Emin.* 22362! 22361!, (ARTH 11802, 11801).

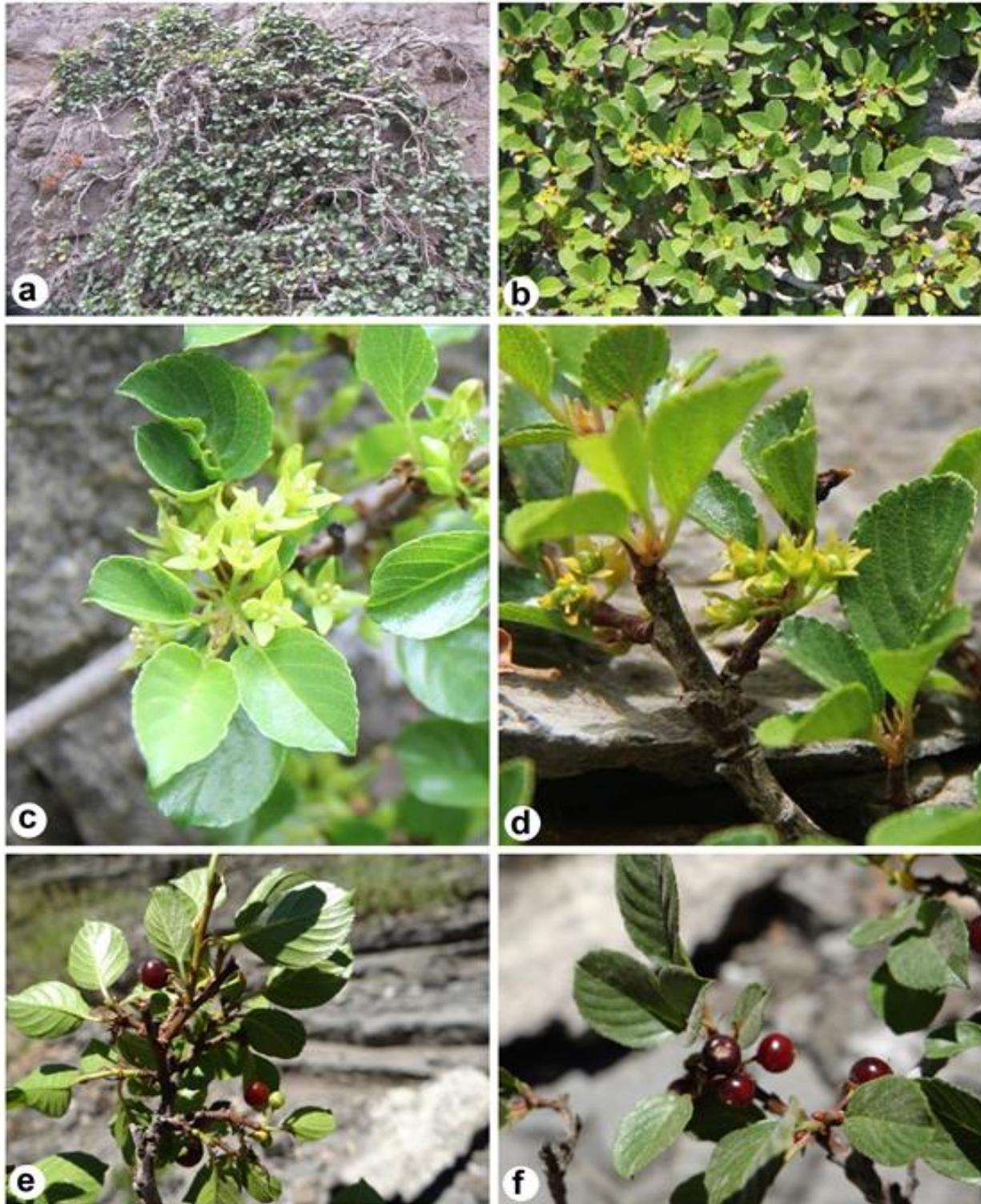


Figure 1. General morphology of *Rhamnus depressa*. a: habitus, b: ascending branches, c: male flowers, d: female flowers, e, f: leaves and fruits

3.2. Micromorphology

Leaf surface sculptures are wavy in the both parts and epidermal cell walls are not distinct. Periclinal surfaces of upper parts are with many small and irregular protuberances and epicuticular waxes. On the other hand, abaxial leaf surfaces are densely covered with epicuticular scaly structures/particles especially among the irregular elevations. Stomata also hide with these elevations. Sparsely simple trichomes can be visible in the lower midrib part of leaf with different length (Figure 2).

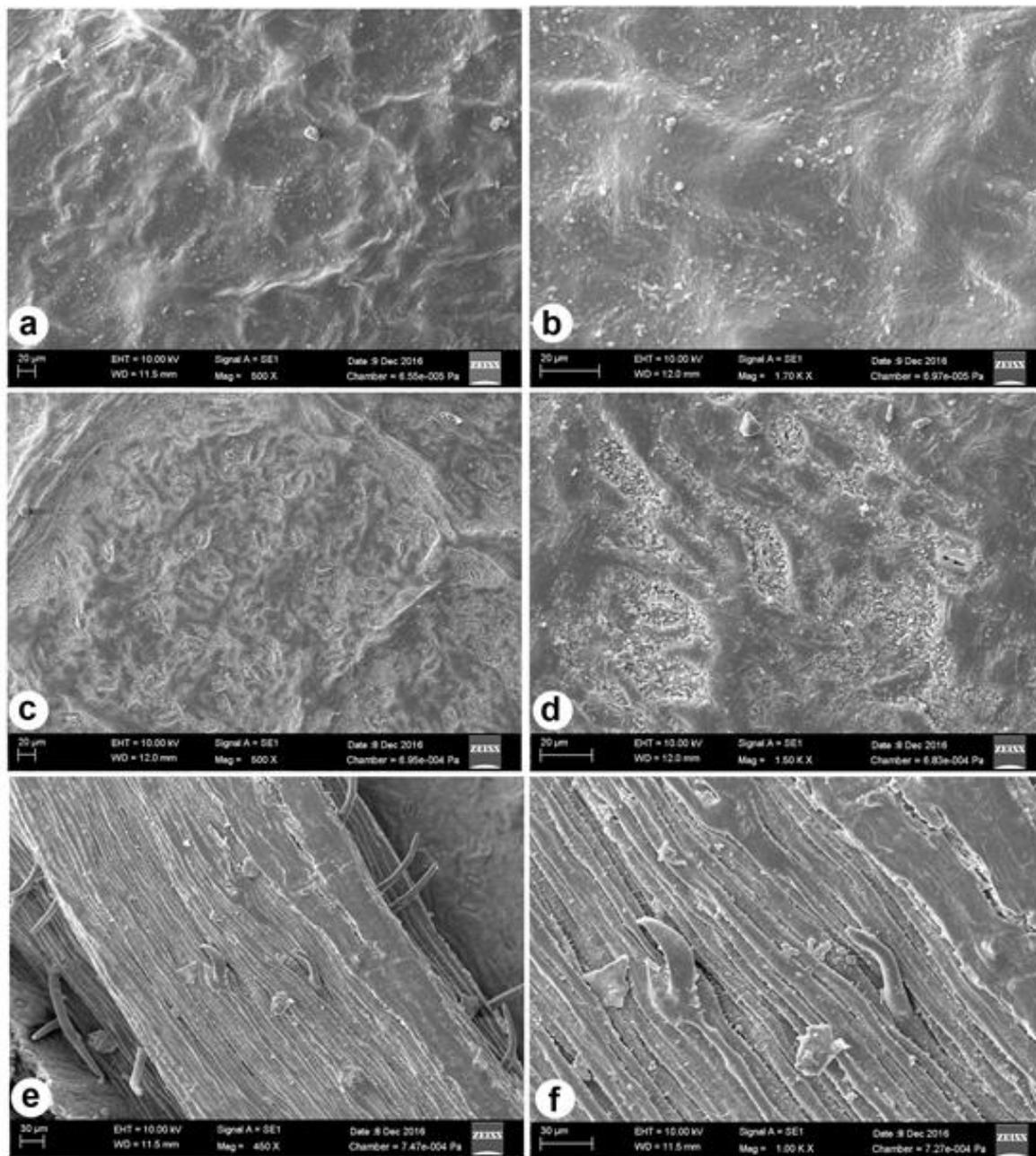


Figure 2. Leaf SEM micrographs of *R. depressa*. a, b: Adaxial surface, c, d: Abaxial surface, e, f: midrib

3.3. Wood anatomy

Wood diffuse to semi-ring porous. Growth ring boundaries are distinct with partially gelatin-free fiber tissue in latewood zone and relatively larger earlywood vessels (Figure 3a). Vessels mostly diagonally and in dendritic patterns, partially radial and tangential small/short multiples, and in clusters, or in solitary forming together with vasicentric vascular tracheids, rounded to angular in cross-section. Earlywood vessels tangential diameter 35 μm and radial diameter 40 μm , latewood vessels tangential diameter 19.8 μm and radial diameter 24.9 μm , vessels ca. 157.2 / mm^2 , vessel member length 257.2 μm (Table 2). Perforation plates simple. Vessel pits alternate, round to oval, with slit-like apertures. Vessels and vasicentric vascular tracheids with helical thickening. Libriform fibres 560 μm long, 14.32 μm wide, thin- to thick-walls 4.43 (3-5.5) μm , gelatinous (Table 2). Axial parenchyma apotracheal and paratracheal marginal bands. Rays heterocellular, composed of slightly upright and square marginal cells and mostly procumbent cells (Figure 3b). Rays 8 (7-12) / mm , uniseriate, biseriate and multiseriate. Uniseriate ray height 125.4 μm and 5 (2-11) cells, biseriate ray height 163.7 μm , multiseriate ray height 276 μm (max. 33 cells) and multiseriate ray width 58.7 μm , 2-5 (6) cells (Table 2). Perforated ray cells present (Figure 3c). Crystals solitary in ray cells (Figure 3d).

Table 2. Wood anatomical characteristics of *R. depressa* compared with *R. microcarpa*

Characteristics	<i>R. depressa</i>	<i>R. microcarpa</i> [17]
Tangential diameter of earlywood vessel (μm)	35 (15-60)	38.8 (20-75)
Radial diameter of earlywood vessel (μm)	40 (25-60)	53.9 (30-80)
Tangential diameter of latewood vessel (μm)	19.75 (10-30)	22.8 (15-32.5)
Radial diameter of latewood vessel (μm)	24.9 (10-47.5)	27.3 (15-50)
Number of vessels per mm^2	157 (124-210)	127 (95-156)
Vessel member length (μm)	257.2 (185-395)	242 (145-300)
Libriform fibre length (μm)	560 (400-700)	584.8 (370-850)
Libriform fibre width (μm)	14.31 (10-16)	11.55 (9-15)
Libriform fibre thickness of cell walls (μm)	4.42 (3-5.5)	3.75 (3-6)
Multiseriate ray height (μm)	276 (145-480)	252.8 (90-515)
Multiseriate ray width (μm)	58.7 (37.5-90)	49 (22.5-75)
Biseriate ray height (μm)	163.7 (90-335)	120.6 (55-225)
Uniseriate ray height (μm)	125.4 (45-280)	98.7 (40-230)
Uniseriate ray height (cell)	5 (2-11)	5 (2-13)
Number of rays per mm	8 (7-12)	10 (7-12)

3.4. Leaf anatomy

3.4.1. Petiole

Petiole is more or less rounded shape in outline. Vascular bundle is open arc-shaped. Epidermal cells are narrow or ovate. Single vascular bundle comprises a wide area in the middle (Table 3). Collenchyma is 3-4 layered and surround of the petiole. Many parenchymatous cells containing druse crystals cover a large area in the surrounding of vascular bundle. This species consists of a big vascular bundle in the middle of the petiole. Trichomes are simple, Uniseriate and unicellular, and cover all petiole, but glandular trichomes are not present. Sclerenchymatous clustered cells are observed in phloem part of vascular bundles. Druses crystals occur in parenchyma cells surrounding the vascular bundles (Figure 4 a, b).

Table 3. Petiole anatomical characteristics of *R. depressa* compared with *R. microcarpa*

Characteristics	<i>R. depressa</i>	<i>R. microcarpa</i> [17]
Xylem thickness	163.09±6.33	219.29±05.93
Phloem thickness	222.16±6.16	232.01±5.93
Vascular bundle thickness	481.15±10.19	623.93±35.56
Vascular bundle breadth	635.71±21.54	776.91±24.12
Trachea size	16.91±0.63	19.57±0.49
Cortex thickness	245.01±8.78	247.97±34.67
Collenchyma thickness (upper surface)	55.07±3.36	77.87±13.03
Collenchyma thickness (lower surface)	49.38±11.27	55.37±11.51
Petiole thickness	970.96±14.84	1236.41±11.20
Petiole breadth	1185.70±8.38	1432.06±8.53

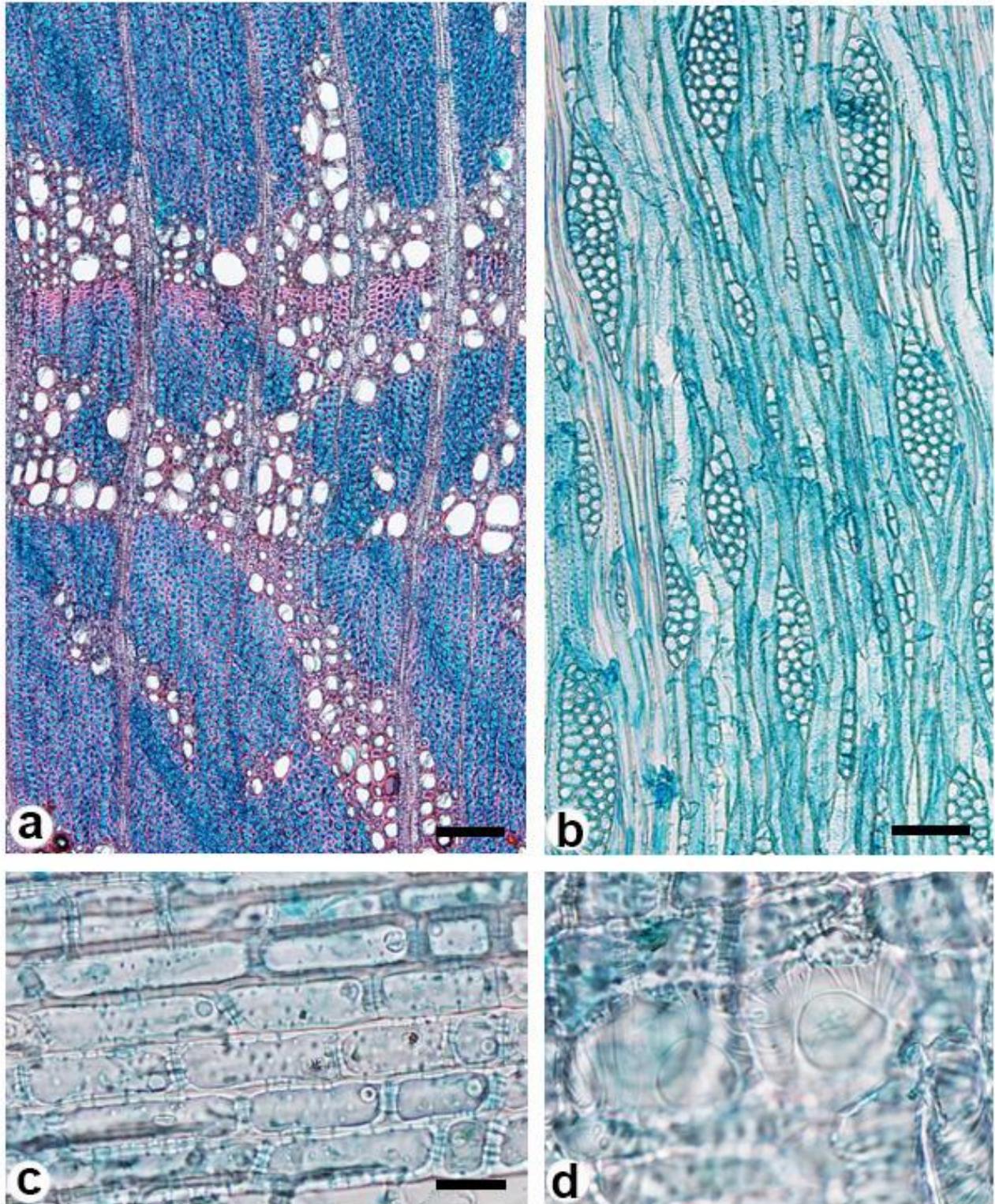


Figure 3. Wood anatomy of *R. depressa*. a: diffuse to semi-ring porous wood, vessels diagonally and in dendritic patterns, partially radial and tangential multiples, and in clusters, or in solitary, b: uniseriate, biseriate and multiseriate, heterocellular rays, c: crystals in ray cells, d: perforated ray cells. Scale bars: a, b: 100 μ m; c: 20 μ m.

3.4.2. Midrib

It is semi-circle, slightly sulcate and covers a large area. Adaxial part is concave, while abaxial one is convex. Under the upper epidermis, several layers of collenchyma cells are observed, but not in the lower epidermis. One or sometimes two collateral and arc -shaped vascular bundles cover a large area. Many sclerenchymatous cells can be visible in phloem part of vascular bundles (Figure 4 c, d).

Table 4. Leaf anatomical characteristics of *R. depressa* compared with *R. microcarpa*

Characteristics		<i>R. depressa</i>	<i>R. microcarpa</i> [17]
	Upper epidermis length	14.33±0.53	13.27±0.40
	Upper epidermis width	20.26±2.81	18.88±1.43
	Lower epidermis length	13.65±0.20	13.40±0.55
	Lower epidermis width	23.57±0.29	24.44±1.40
	Midrib mesophyll thickness	532.72±8.19	659.23±14.77
	Midrib mesophyll breadth	657.23±19.80	775.57±22.43
	Lamina mesophyll thickness	168.16±2.44	181.84±1.45
	Trachea size	11.94±0.22	12.58±0.11
	Xylem thickness	149.25±3.15	190.06±4.83
	Phloem thickness	178.73±2.81	204.43±4.84
	Vascular bundle thickness	332.27±5.77	396.38±9.55
	Vascular bundle breadth	474.52±9.59	552.89±14.36
Adaxial surface	Cuticle thickness	7.60±0.06	5.57±0.15
	Stomatal length	36.64±0.69	0
	Stomatal index	4.71±0.34	0
	Stomata number per mm ²	40±6.31	0
Abaxial surface	Cuticle thickness	6.54±0.11	6.20±0.15
	Stomatal length	39.23±0.36	27.68±0.48
	Stomatal index	3.08±0.41	11.57±0.84
	Stomata number per mm ²	32±7.99	30.8±4.22

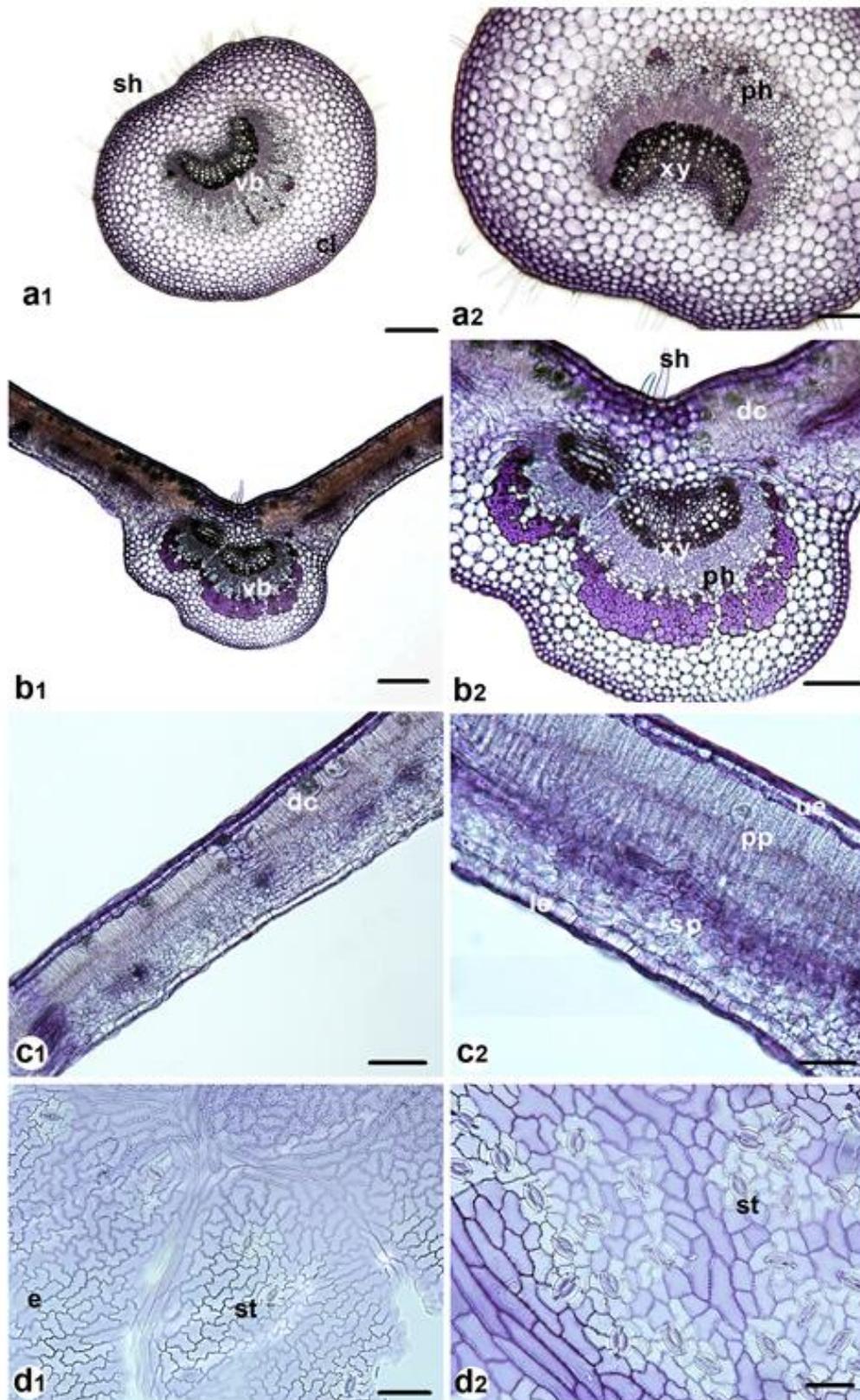


Figure 4. Leaf anatomy of *R. depressa*. a-c: cross section, d: peripheral section (1- adaxial surface, 2- abaxial surface). a: petiole, b: midrib; c: lamina. cl: collenchyma, dc: druse crystals, le: lower epidermis, ph: phloem, pp: palisade parenchyma, sh: simple hair (trichome), sp: spongy parenchyma, ue: upper epidermis, vb: vascular bundle, xy: xylem. Scale bars: 200 μm (a1, b1), 100 μm (a2, b2, c1), 50 μm (c2, d).

4. Conclusions and discussion

It was emphasized that these two species, which are very similar to one, should be examined in detail morphologically. It has been noted that the *R. depressa* differs from the *R. microcarpa* only with very small elliptical leaves with fewer veins on either side without any measurements [5]. In our study, we determined that some morphological characters are differentiating *R. depressa* from *R. microcarpa* [17] (Table 1). Especially, petiole length, leaf sizes, and numbers of bud scale, seed, and leaf veins have distinguished this species distinctly.

Hui et al. [21] studied micromorphological leaf features of 41 species and four varieties belonging to the Rhamnaceae family and undulate leaf surfaces were determined. According to surface ornamentation, smooth and striate adaxial epidermal cells or epidermal cells having scaly structures were also reported. It has been also mentioned that leaf micromorphological characteristics could be used at the species or subspecies level classifications in Rhamnaceae. In addition, leaf micromorphological characteristics of *R. microcarpa* have been reported by Eminağaoğlu et al. [17]. Leaf micromorphological results of this study are in accordance with previous reports mentioned above.

In some of earlier studies, it has been reported that the wood anatomy features of *Rhamnus* species are very similar and cannot be clearly differentiated [10, 15], while in others, in some species can be distinguished by the ray width and height, or marginal axial parenchyma [11, 14, 16]. Schweingruber [11] stated that *R. alaternus* can be distinguished by their short rays and *R. alpinus* by their wide and tall rays from other *Rhamnus* species. The average uniseriate ray height is 98.7 µm, the average biseriate ray height is 120.6 and the average multiseriate ray height and width are 252.8 µm (max. 46 cell) and 49.0 µm (2-7 cells, max. 8) for *R. microcarpa* [17]. In this study, the average uniseriate ray height, biseriate ray height, multiseriate ray height, and width were detected as an average of 125.4 µm, 163.7 µm, 276.0 µm (max. 33 cell), and 58.7 µm (2-5 cell, max. 6) in *R. depressa*, respectively. Although the maximum width and height of the multiseriate rays was found to be higher in *R. microcarpa*, it can be said that *R. depressa* has longer and wider rays when the other average ray values are considered. The multiseriate ray width of five endemic *Rhamnus* species in Turkey was found as 1-3 (max. 3-5) [14]. It has been noted that the height of the rays tends to change during ray ontogeny, that many of its features may vary in ways that limit their taxonomic usefulness, but differ significantly in quantitative in different species [33]. Since rays' height varies depending on ontogeny, it has been reported that the density of the ray is more effective and useful than the height of the ray in the identification of wood [33]. The mean ray density of *R. depressa* (8) was lower than the other taxa (9-16) in Turkey [12, 14, 15, 17].

There is no difference in the length of the fibers and the vessel members in *R. depressa* and *R. microcarpa* (Table 2). The tangential and radial diameters of the vessels of *R. depressa* are smaller than *R. microcarpa*, but the number of vessels per mm is higher [17]. Vessel density and diameter are two of three quantitative vessel features that are incorporated into the mesomorphy ratio. It has been reported that the calculated low mesomorphy value below 75 indicates xeromorphy [34, 33]. *R. depressa* is a xerophyte plant and is in the form of dwarf shrub wrapped on rocks, like *R. microcarpa*. The mesomorphy value of the *R. depressa* is 44.19. Though the mesomorphy value of *R. depressa* is lower than *R. microcarpa* (59.07) [17], it is higher than the Mediterranean species in Turkey (11-23) [14, 15]. The values calculated by Merev [12] for the species growing in the Eastern Black Sea Region are quite high.

In previous studies, petiole anatomical characteristics of *Rhamnus microcarpa* which is closely related *R. depressa* were reported by Eminağaoğlu et al. [17] from Turkey and *R. wightii* Wight & Arn. by Shisode and Patil [22] from India. In accordance with our results they reported horseshoes petiole, arc-shape vascular bundle and druses crystals in parenchyma cells. Efe et al. [19] also reported orbiculate petiole for *Rhamnus pichleri* Schneider and Bornm. ex Bornm. In addition, Efe et al. [19] observed secretory cavities in *R. pichleri*. On the other hand, the secretory cavities mentioned do not observed in our studies species. We determined the dorsiventral mesophyll (bifacial leaf) for *R. depressa*. Similar to our findings, Varone and Gratani [35] investigated leaf expansion of *R. alaternus* L. and reported bifacial leaf mesophyll for this species.

Unicellular trichomes were revealed by Shisode and Patil's report [22] in *R. wightii* and by Serdar et al. [20] in two subspecies of *Frangula alnus*. Furthermore, it was also mentioned from stellate scales in *Pomaderris apetala* Labill. by Shisode and Patil's report [22]. We also determined many epicuticular scales in the abaxial surface of leaf of *R. depressa*.

In literature, hypostomatic leaf type is usual in most of the species. Efe et al. [19] reported this type leaf in most of their studied species, except for *Rhamnus thymifolius* Bornm. with amphistomatic leaf. From previous reports, Hui et al. [21] found that most species in the family Rhamnaceae have hypostomatic type, with small exceptions (*Colubrina asiatica* (L.) Brongn). We found the amphistomatic leaf with stomata in the both surfaces of *R. depressa*, differently from closely related species *R. microcarpa*.

Efe et al. [19] reported calcium oxalate crystals in the palisade cells of five *Rhamnus* species. Shisode and Patil [22] reported these types' crystals in *R. wightii*. Serdar et al. [20] also mentioned different amount of druse crystals in petiole of *Frangula alnus* Mill. The authors also reported secretory cavities in *R. wightii* and also some other species in the family Rhamnaceae. Our results are in agreement with these reports except for secretory cavities.

This study reveals several morphological and anatomical characteristics that can be used in differentiating *R. depressa* and *R. microcarpa*, i.e petiole length, leaf sizes, and numbers of bud scale, seed, and leaf veins, ray

characteristics in wood, amphistomatic stomata, horse shoes vascular bundle in the petiole, one vascular bundle or two vascular bundles with sclerenchymatous cells in phloem part, as well as epidermis micromorphological characters.

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