Taiwania 62(3): 321–330, 2017
DOI: 10.6165/tai.2017.62.321

Comparative study of autecological, morphological, anatomical and karyological characteristics of *Acanthophyllum ejtehadii* Mahmoudi & Vaezi (Caryophyllaceae): a rare endemic in Iran

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(Manuscript received 8 December 2016; accepted 28 May 2017; online published 28 July 2017)

ABSTRACT: Characterizing traits of a species in its habitat is substantial for planning a practical conservation program. *Acanthophyllum ejtehadii* Mahmoudi & Vaezi (Caryophyllaceae) is a recently established endemic species for flora of Iran and has a narrow non-conserved natural habitat in Radkan region of Chenaran, Razavi Khorasan Province. The soil and climate of Radkan was studied and the ecological, morphological, anatomical, and karyological aspects of *A. ejtehadii* were investigated to obtain a comprehensive knowledge about this species and its natural growth conditions. Field observations were performed during the growing seasons in 2014–2015 and 29 vegetation samples were collected as data. Results showed that this plant grows in mound-like sites on clay-loam soils at mean elevation 1279 m.a.s.l. in arid climate. *Acanthophyllum ejtehadii* is a thorn-cushion form chamaephyte plant. This Plant grow gradually in early-January, the flower unfolds in early-June and the matured seeds are produced in mid-July. *A. ejtehadii* is a diploid (2n=2*x* =30) species and has homogenous karyotype. Having unique morphological and anatomical adaptations such as expanded surface roots, reduced leaf area and thickened cuticle, this plant grows successfully in harsh environments. These mechanisms are specific to this specific kind of *Acanthophyllum* species. The Speciation time of this plant was estimated not more than 200 thousand years ago and if Radkan is preserved from anthropogenic disturbance, this species could expand its distribution area.

KEY WORDS: *Acanthophyllum ejtehadii*, Anatomy, Autecology, Karyology, Morphology.

INTRODUCTION

Characterizing traits of a species in its habitat is substantial for planning a practical conservation program. These types of studies make a distinct discipline in ecology, called Autecology. Autecology studies mainly concern the relationship of a species to biotic and abiotic conditions in its habitats (Pianka, 2008; Köppler and Hitchmough, 2015).

Different properties of a species are called its traits. These traits are representatives of the learnt behaviors in response to different events through the evolutionary history of that species. Autecology gives us the precise information about how a plant species responds to environmental changes (Köppler and Hitchmough, 2015).

Iran is one of the large countries in South Western Asia with an area of approximately 1640000 square kilometers. Because of the different climatic conditions, caused by high elevation that surrounds the central parts of Iran, different ecosystems with unique features are created in Iran (Ghahreman and Attar, 1999). These unique natural conditions make Iran a homeland for about 8000 plant species, among them 1727 species are endemic (Jalili and Jamzad, 1999). Although there are a lot of endemic plant species in Iran, unfortunately a lack of knowledge about their characteristics and habitats made them vulnerable to blind human acts. It is necessary to study endemic species traits for planning sustainable usage and managing their natural habitats for conservative goals.

Genus *Acanthophyllum* belonging to Caryophyllaceae has 80-90 species worldwide (Pirani *et al.*, 2014). *Acanthophyllum* species are dwarf shrubs, perennial, with thorn shaped leaves and white or pink flowers (Schiman-Czeika, 1988). They are mainly distributed in arid lands and foothills of temperate regions (Heywood, 1985) and in the main part of steppes and subalpine vegetation in Central and Southwest Asia (Zohary, 1973). According to Takhtajan (1986), all species of this genus grow in Irano-Tournian floristic region. NE of Iran, Afghanistan and Turkmenistan are speciation and diversification zones of this taxon.

Acanthophyllum ejtehadii Mahmoudi & Vaezi is a recently recognized species for flora of Iran whose natural habitat is limited to the northeast of Iran (Mahmoudi-Shamsabad *et al.*, 2012). Molecular phylogenetic analysis supports that this species is a distinct species in Caryophyllaceae (Pirani *et al.*, 2014).

Heretofore there is no autecological research on *A. ejtehadii*. In this study, ecological, morphological, anatomical, and karyological properties along with soil texture, climatic and physiographical properties of the only habitats of *A. ejtehadii* were recorded, measured and described. This study aimed to present a reliable

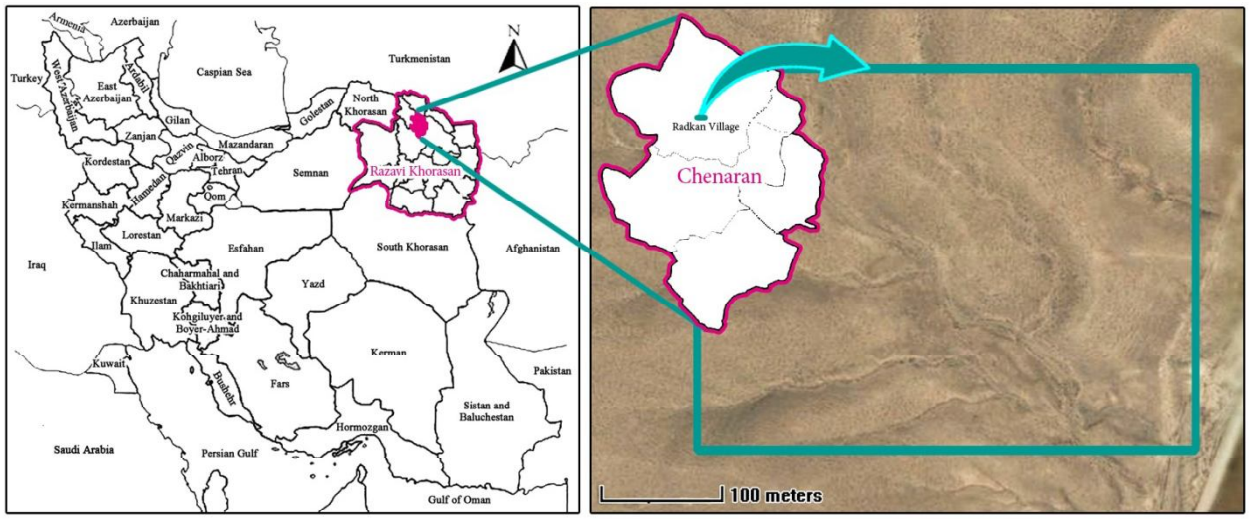


Fig. 1. Geographical location of Radkan region.

comprehensive data about this species so as to be of use to its conservation.

MATERIALS AND METHODS

Study area

Acanthophyllum ejtehadii grows in Radkan Region in the northwest of Chenaran city, Razavi Khorasan Province. The distribution of the *A. ejtehadii* is between 36°50′5.4″ and 36°50′12.9″ north latitude and between 59°00′56.4″ and 59°01′10.1″ east longitude (Fig. 1). This area geologically belongs to Kopet-Dagh zone, Shorije formation. Lithological studies have reported shale, gypsum and sandstone with Neocomian age construct as the main bedrock of this area (Moussavi-Harami *et al.*, 2004).

Climate and soil properties of Radkan

In order to estimate climatic parameters of the study area, POWER's Climatology Resource for Agroclimatology data for a period of 20 years (1994-2014) were downloaded. De Martonne Aridity Index (Oliver, 2005) and Emberger method (Villeneuve, 1980) were used for climatic classification of Radkan. Using the same data, the Ombrothermic Diagram of this area was also drawn.

Physiographical characteristics of the study area including the elevation above sea level, slope aspect and slope degree for *A. ejtehadii* in the growing region were observed and recorded.

Three soil samples near *A. ejtehadii* individuals from 0-20 cm depth were collected. Soil properties including pH, Electrical Conductivity (EC), texture, Nitrogen, Phosphorus and Potassium content and Calcium Carbonate percentage were investigated in the Pedology Laboratory of Agriculture Faculty of Ferdowsi University of Mashhad, Iran.

Autecological study

Field sampling for autecological study of *A. ejtehadii* were performed in the 2014–2015 growing seasons. Considering the sparse distribution of this species, 29 selective plots with 1 square meters area, including one individual in the center of each plot, were used.

In each sampling plot, the canopy diameter and cover percentage, phenological periods, revitalization strategies and life form (Raunkiær, 1943) of *A. ejtehadii* were recorded. The companion plant and insect species were also collected and identified through standard keys.

Three random flowering individuals were selected for observing the root elongation pattern, root diameter and root depth. These individuals were also used for further morphological and anatomical studies.

Morphological study

Morphological characteristics of this species were measured in the Plant Ecology Research Laboratory of Ferdowsi University of Mashhad. Twenty six quantitative and seventeen qualitative characters were selected for morphological description of *A. ejtehadii*. Quantitative characters were pictured by Dino-Lite Plus AM313T Camera and measured by DinoCapture 2.0 software.

In terms of Morphological structure, Mahmoudi *et al.* (2012) have categorized this species as closely related to *A. diezianum*. In the present study, the morphological characteristics of *A. ejtehadii* was compared with *A. diezianum*, *A. pachystegium* and *A. lilacinum*.

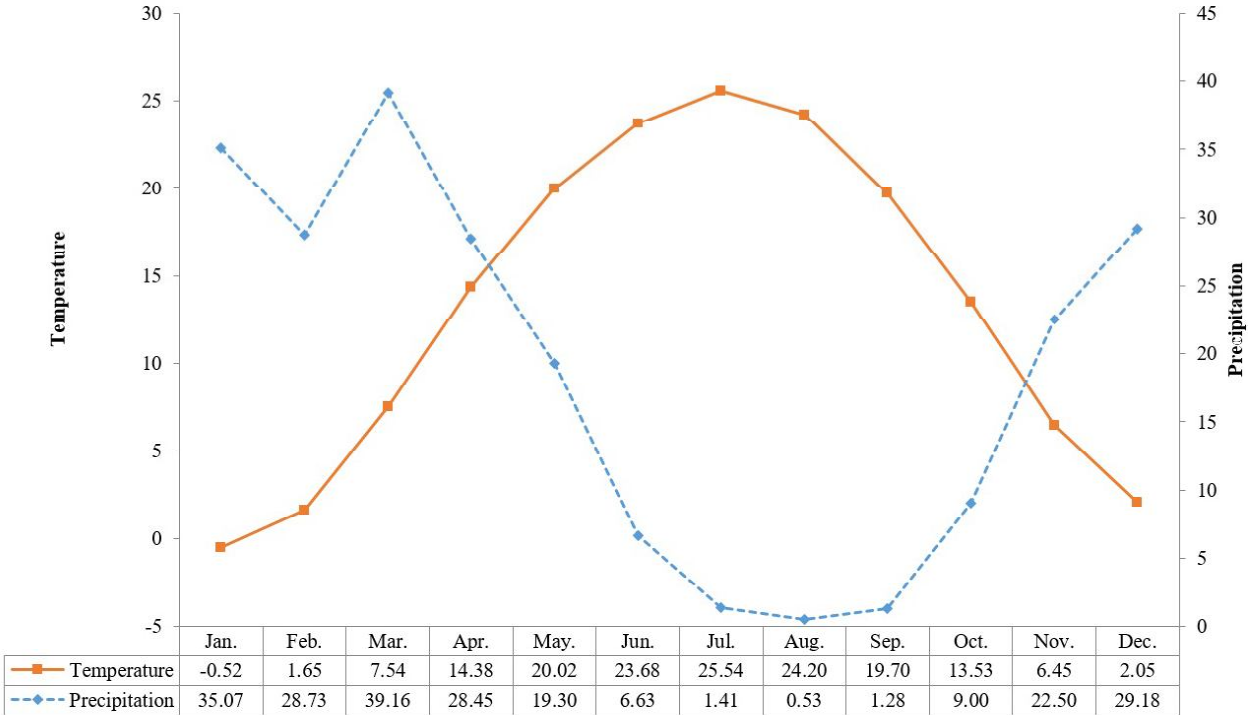
Anatomical study

In this study, anatomical characteristics of leaf, stem and inflorescence of *A. ejtehadii* (19 characters) were measured.

For softening the woody parts of this plant, selected

Table 1. Soil parameters of Radkan region

Habitat	Soil texture	EC (ds/m)	pH	P (ppm)	K (ppm)	N (ppm)	$CaCo_3$
Radkan	Clay Loam	0.48 ± 0.06	8.013 ± 0.05	6.53 ± 1.68	403 ± 10	< 0.1	38 ± 1.73



	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Temperature	-0.52	1.65	7.54	14.38	20.02	23.68	25.54	24.20	19.70	13.53	6.45	2.05
Precipitation	35.07	28.73	39.16	28.45	19.30	6.63	1.41	0.53	1.28	9.00	22.50	29.18

Fig. 2. Ombrothermic Diagram of Radkan region, 20 years (1994-2014) data were used for drawing this diagram. Drought season begins in late April and lasts till October.

organs were immersed in a mixture of 96% alcohol, water and glycerine in volume proportions 1:1:3 for one month. The softened organs were then fixed in Formalin-Acetic Acid-Alcohol (FAA 1:1:18 v/v). Semi-thin cross sections were manually cut and were then stained by Safranin-Fast Green protocol (Gerach, 1977). For recording leaf epidermis properties of A. ejtehadii, thin-layer crosses of leaves' inferior surface were used. The prepared crosses were observed using a light microscope (Olympus BH-2) connected to a digital camera (Dino-Eye Am 423) for picturing. Anatomical features were measured by DinoCapture 2.0 software.

Karyological study

Hand-cut cross sections of radicles (about 1 cm in length) were used for karyological study according to Muller *et al.* (1991). The fixed slides were observed with Olympus Light Microscope BX-50 and Dino-Eye AM423 was used to picture the slides. Measurements were performed by DinoCapture 2.0. Software. Chromosomal sorting for the karyotype drawing was done in Adobe Photoshop CS5.

For karyological analysis and determination of A. ejtehadii karyotype symmetry, five cells in the Metaphase stage were selected and the number of chromosomes and karyotype features including chromosomal formula, long and short chromosomal arm length (L & S), chromosome length (L+S), short/long arm ratio (L/S) and total form percentage (TF %) were calculated through Huziwara (1962) protocol.

RESULTS

Climatic and soil properties of Radkan area

The natural habitat of *A. ejtehadii* is a mound-like region with an average 15° slope and a mean elevation of 1279 meters above the sea level. Average annual precipitation and temperature of Radkan is 221.23 mm and 13.18°C respectively. This area has arid and cold-arid climate based on De Martonne and Embereger climatic classifications. The Ombrothermic diagram of Radkan is shown in Fig. 2.

Radkan's soil consists of 30% clay, 33% silt and 37% sand grains. Therefore, it can be classified as a clay loom texture based on the United States Department of Agriculture soil classification system (2014). The other assessed soil parameters are noted in Table 1.

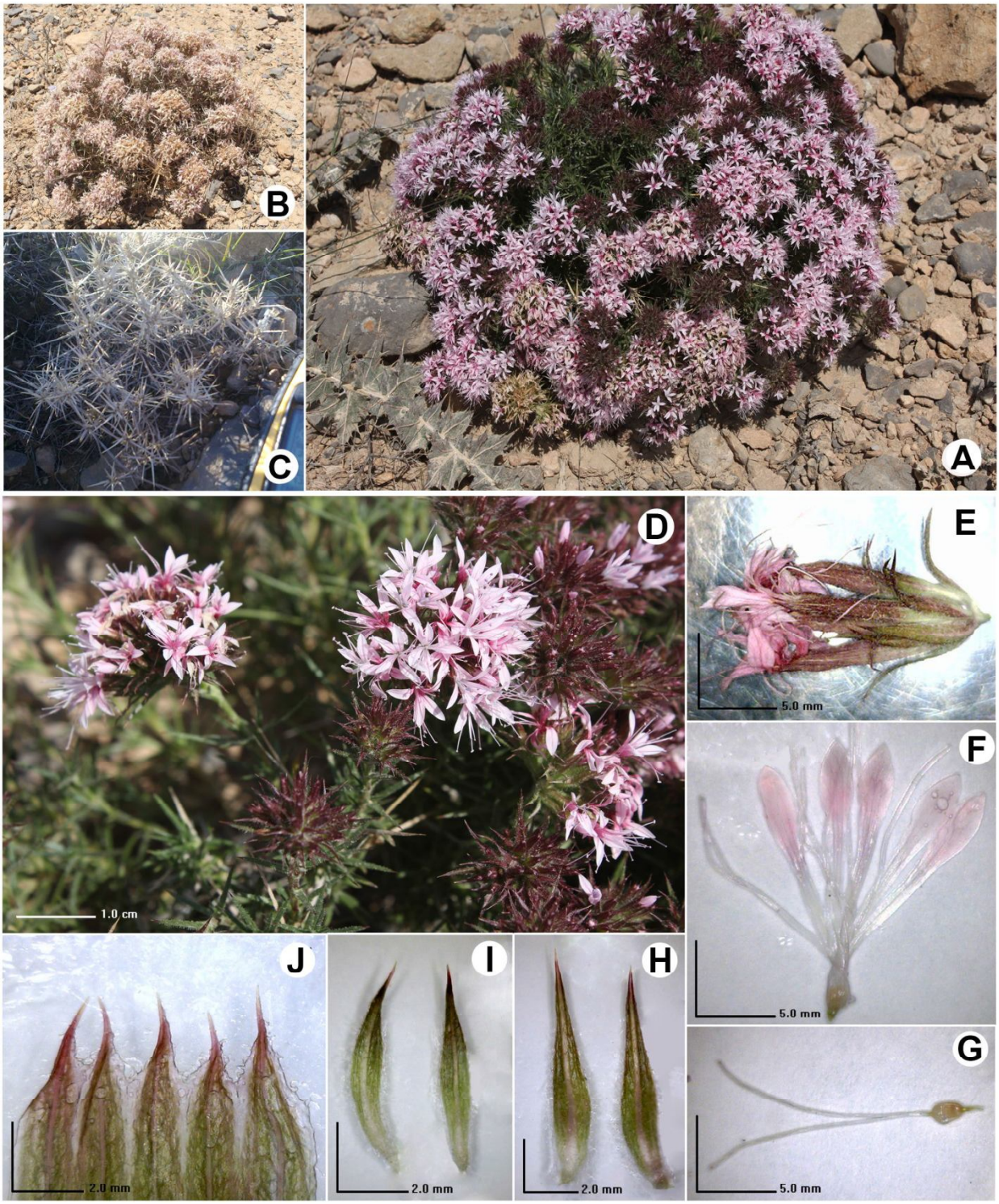


Fig. 3. *Acanthophyllum ejtehadii* Mahmoudi & Vaezi in Radkan area. **A**-**C** Phenological period **A**. Flowering, **B**. Developing the mature seeds **C**. Dormant phase. **D**-**J** Morphological characteristics. **D**. Flowers and inflorescence, **E**. Cyma, **F**. Petals, **G**. Ovary, **H**. Bracts, **I**. Bracteole and 7- Calyx teeth.

Autecological description of *A. ejtehadii*

The vegetative growth of *A. ejtehadii* begins in early January and it lasts till May. This plant reaches its flowering peak in early June. Seed development begins at late June and mature seeds are formed in mid-July. At the same time as seed dissemination stage, leaves abscission of individuals begin and plants gradually become dormant (Fig. 3).

Table 2. 49 Morphological characteristics of ***A. ejtehadii***. Quantitative characters are reported in µm (except for plant height which is reported in cm) units.

No.	Characters	Mean ± SD	No.	Characters	Mean ± SD
1	Plant growing shape	Cushion-shape	25	Bract length	8.84 ± 1.06
2	Plant height	7.4 ± 2.00	26	Bract width	1.22 ± 0.10
3	Flowering branch height	26.69 ± 3.30	27	Bracteole length	7.06 ± 0.87
4	Stem color	Pallid or Gray	28	Bracteole width	0.88 ± 0.10
5	Internodes length	4.41 ± 0.48	29	Petals extra Calyx length	4.35 ± 0.63
6	Phyllotaxy of leaves	Opposite	30	Calyx shape	Tubular
7	Leaves shape	Lanceolate	31	Calyx length	10 ± 0.89
8	Leaves length	15.13 ± 1.74	32	Calyx width	1.64 ± 0.11
9	Leaves width	1.16 ± 0.11	33	Number of calyx teeth	5
10	Types of inflorescence	Dichasial Cymes	34	Calyx teeth size	Unequal
11	Inflorescence diameter	23.83 ± 3.70	35	Calyx teeth shape	Narrow Triangular
12	Number of cyma partialis in inflorescence	14 ± 2.00	36	Calyx teeth length	1.61 ± 0.19
13	Number of flower in cyma partialis	3	37	Calyx mucronum length	0.63 ± 0.09
14	Lower floral leaves spreading	Horizontal	38	Number of petal	5
15	Superior floral leaves spreading	Erect	39	Petal's neak shape	Entire or Sharp
16	Principal inflorescence peduncle length	4.51 ± 1.50	40	Petal's colour	Pink
17	Flower pedicel length	0.77 ± 0.14	41	Petal length	14.15 ± 0.72
18	Floral leaves shape	Lanceolate	42	Petal width	1.70 ± 0.13
19	Floral leaves length	Erect	43	Number of Stamens	10
20	Floral leaves width	1.62 ± 0.20	44	Number of ovules in ovary	4
21	Bracts spreading	Erect	45	Filament length	14.30 ± 0.76
22	Bract's color at neck	Purple	46	Style length	13.98 ± 1.11
23	Bract's color at base	green	47	Hairs on stem	Covered with short glandular and unicellular hairs
24	Bract shape	Acicular	48	Hairs on leaves	Covered with short glandular hairs
25	Bract length	8.84 ± 1.06	49	Hairs on calyx	Covered with short glandular, unicellular and multicellular hairs
26	Bract width	1.22 ± 0.10			

According to Raunkiær plant life-form (1934), *A. ejtehadii* is a chamaephyte plant. Average canopy of this taxon is 7.65% in 1 square meter area, with 7–27 cm diameter in flowering individuals. This species distributes by wind dispersion of mature seeds. It must be noted that the dormant individual regenerates in every growing season.

Red root of *A. ejtehadii* extraordinary grows in a shallow system. Tap root grows 5–7 centimeters downward and then divides into 2–3 parts; these parts form the sinker roots, grown at radius of 16–50 cm, near soil surface.

27 plant species belonging to 18 families were observed with *A. ejtehadii* in the sampled plots. Among these species, *Rosa persica* Michx. ex Juss., *Prunus spinosissima* Franch and *Artemisia scoparia* Waldst were observed in all of the plots. *Thrips* sp. (Thysanoptera) was abundantly observed in *A. ejtehadii* flower calyx.

Morphological characteristics

Acanthophyllum ejtehadii is a cushion form dwarf shrub with 4–11 cm heights in flowering individuals. Its stems are gray or light-gray, covered by globular and unicellular trichomes, with internodes of 4±0.5 cm in length. Leaves are opposite, lanceolate with 1–1.3 mm in length (Fig. 3). Morphological properties are described in Table 2.

The bracts in *A. ejtehadii* and *A. diezianum* are acicular and erect and the floral leaves are lanceolate (the lower floral leaves are horizontal, while the upper floral leaves are erect). The sprouts in *A. ejtehadii* branch from the middle of the stem while in the *A. diezianum* the sprouts branch from the base as well. Moreover, in *A. ejtehadii* the stem, leaves, peduncle and calyx are covered with short and long glandular hairs and single-celled and multicellular simple hairs, whereas the same organs in *A. diezianum* are covered with only multicellular simple hairs. In *A. lilacinum* and *A. pachystegium*, the floral leaves are subulate (the lower floral leaves and the upper ones are semi-horizontal and curved, respectively) and the bracts are subulate and curved.

Anatomical characteristics (Fig. 4)

Measured anatomical properties of *A. ejtehadii* are described in Table 3. Stem and inflorescence cross

Table 3. Anatomical characteristics of ***A. ejtehadii***. The measured characteristics are reported as Mean ± SD micrometers.

Characters	Stem (µm)	Peduncle (µm)	Leaf (µm)
Length of cross section	959.256 ± 17.76	827.71 ± 10.22	903.84 ± 9.38
Width of cross section	805.21 ± 4.57	589.70 ± 10.05	449.23 ± 16.5
Size of Cuticle	3.93 ± 0.28	3.73 ± 1.31	3.67 ± 0.86
Size of Epidermal layer	12.27 ± 3.42	7.59 ± 1.23	13.21 ± 0.91
Size of Parenchyma layers	17.10 ± 4.96	21.39 ± 8.49	68.17 ± 14.67
Size of Sclerenchyma layers	193.74 ± 22.82	143.97 ± 13.98	333.36 ± 17.54
Length of vascular bundle	466 ± 7.67	440.51 ± 22.73	108.56 ± 14.63
Width of vascular bundle	346.70 ± 9.2	283.94 ± 17.20	42.40 ± 6.58
Size of Phloem	29.47 ± 3.38	22.46 ± 4.70	-
Size of xylem	64.34 ± 8.79	75.86 ± 7.35	-
Length of Pith	380.60 ± 3.93	278.48 ± 27.21	-
Width of Pith	161.73 ± 11.41	100.153 ± 7.179	-

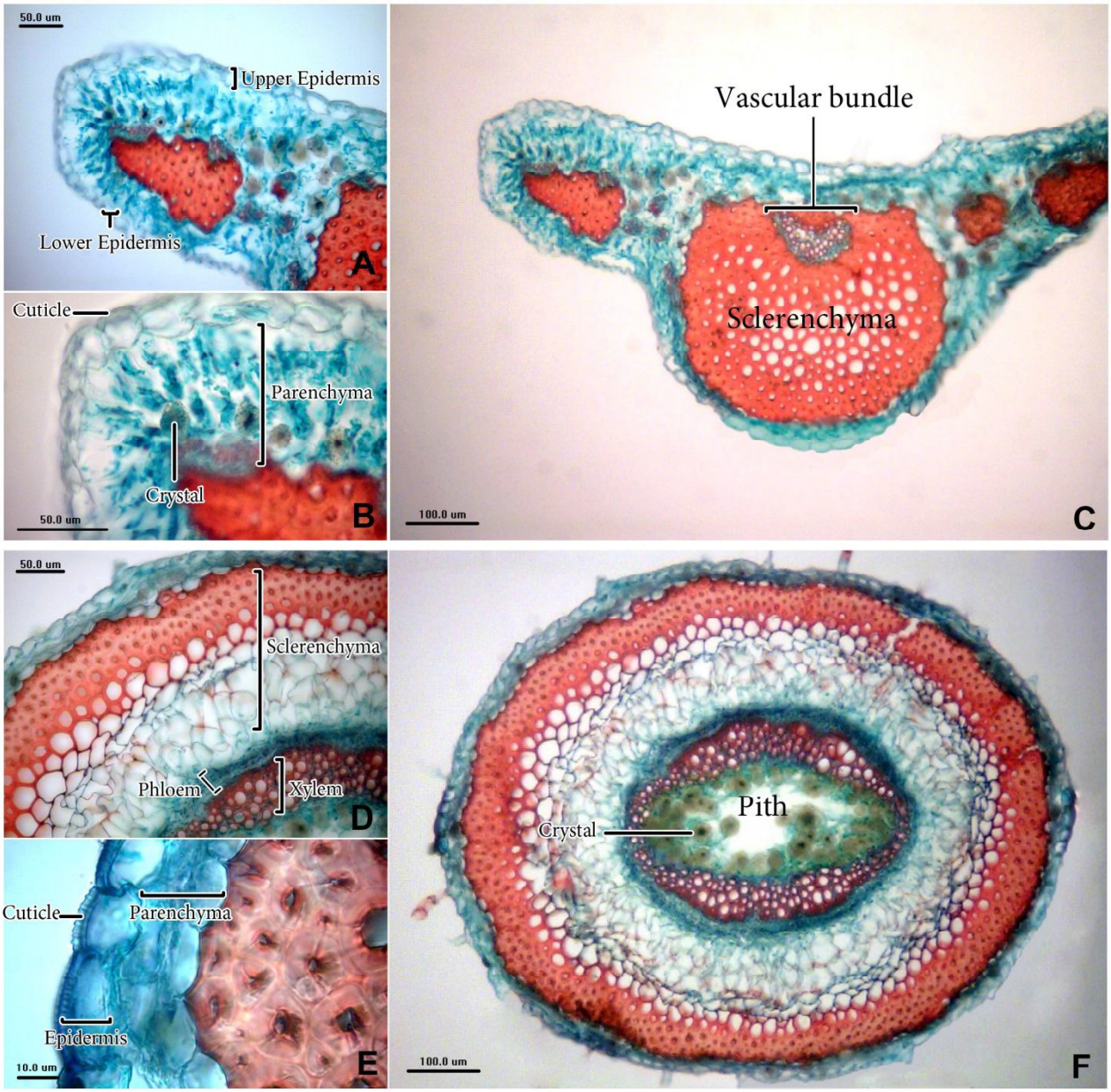


Fig. 4. Anatomical characteristics in Cross-section of ***A. ejtehadii.*** **A-C.** Leaf. **D-F.** Stem.

sections of this species are ellipsoid, both including (from outer to inner) epidermis with thick cuticle, with 2–4 layers of parenchymal cells, sclerenchyma, vascular bundles, and pith with parenchymal cells having calcium oxalate crystals (Fig. 4).

Palisade parenchyma cells with calcium oxalate crystals lie down beneath the epidermis layer in a leaf's cross sections. Sclerenchyma cells with vascular bundles fill the mid area (Fig. 4).

Acanthophyllum ejtehadii's leaf epidermis have 88.48 ± 10 µm length and 49.94 ± 11 mm width, its cell walls are anticlinal curling. Anomocytic stomata of this plant have 30.81 ± 5 µm length and 25.63 ± 3 µm width (Fig. 5).

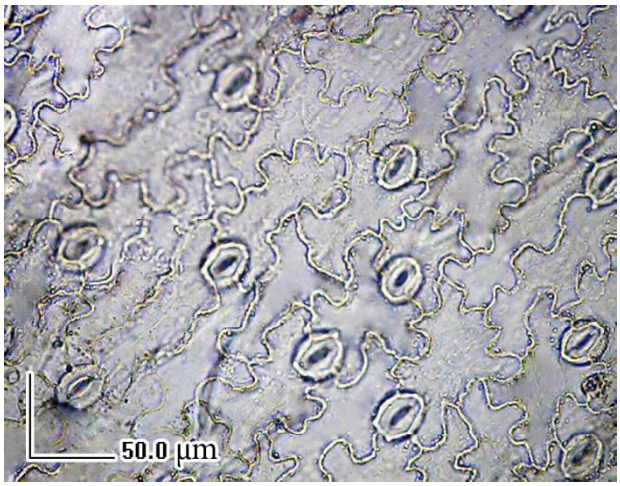


Fig. 5. Lower epidermis of ***A. ejtehadii***. This species has an anomocytic stomata.

Karyological characteristics

Acanthophyllum ejtehadii is a diploid species with $2n=2x=30$ chromosomes and $x=15$ basic chromosome number. Its longest and shortest chromosomes have 2.96 and 2.03 µm length, respectively. The karyotypic formula of this species is 12m + 3sm and TF=43.175 (Fig. 6). Full list of *A. ejtehadii* karyological features are noted in Table 4.

Table 4. Karyological properties of ***A. ejtehadii***. L is the longest arm length, S is the shortest arm length. m is the metacentric and sm is the submetacentric chromosome

Pairs	L (µm)	S (µm)	L + S (µm)	L / S	Type
1	1.50	1.40	2.90	1.07	m
2	1.51	1.30	2.81	1.17	m
3	1.56	1.12	2.68	1.39	m
4	1.48	1.15	2.63	1.29	m
5	1.68	0.92	2.60	1.82	sm
6	1.37	1.20	2.57	1.14	m
7	1.31	1.23	2.54	1.06	m
8	1.29	1.09	2.38	1.19	m
9	1.29	1.03	2.32	1.26	m
10	1.47	0.85	2.32	1.72	sm
11	1.47	0.79	2.26	1.87	sm
12	1.24	0.99	2.23	1.25	m
13	1.26	0.94	2.21	1.34	m
14	1.16	0.97	2.13	1.19	m
15	1.24	0.84	2.08	1.47	m

Discussion

In this study, ecological, morphological, anatomical, and karyological characteristics of *A. ejtehadii*, a recently known species to the world's flora along with the features of its only habitat in the northeast of Iran, were studied.

Radkan drought season begins at late-April and continues for near 6 months till late-October. The mean evaporation rate in drought seasons is high, where the water stress can limit the plant growth. Water stress adversely changes many normal functions of plant species. For successful survival and reproduction, plants must respond to these changes. With two different strategies of drought escape and drought tolerance, plants are adapted to water deficiency (Taiz and Zeiger, 2010; Kooyers, 2015).

Rapid development for efficient grow in wet season is a cost-effective mechanism for escaping harsh conditions of water shortage (Mitra, 2001). Drought escape is the response of *A. ejtehadii* to dry season, with rapid growing and early senescence. It seems that the drought tolerance mechanism is specific to this species as well as the other Acanthophyllum spp.. For instance, flowering stages of A. yasamin-nassehiae Joharchi & Pirani and *A. pachystegium* (grown in Radkan region) occur in late summer (Pirani *et al.*, 2013) and early July (field observations), respectively. Many desert plants use this mechanism to avoid harsh environmental conditions caused by dry seasons (De Micco and Aronne, 2012).

Shortening the life cycle is not the only adaptation of *A. ejtehadii* in arid environments. Reducing leaf surface, thickening cuticle and developing multilayer sclerenchyma are some anatomical features considered as a response to dry environments. Reduction in leaf number and leaf surface are considered as an effective strategy to reduce evaporation from surface of the leaves. (Lobato *et al.*, 2008; Osuagwu *et al.*, 2010). Thickening of Cuticles and mesophyll in response to water stress was also reported in Stipa lagascae (Boughalleb *et al.*, 2015).

Acanthophyllum ejtehadii's root growth system is one of the outstanding specific adaptation mechanisms of this plant in response to its habitats. The primary roots are relatively short and the first order lateral fibrous roots are fairly long and grow near the soil surface. These types of root, which are considered as type II, are also seen in Ferocactus wislizeni (Cannon, 1949). These types of roots allow plants to collect surface waters during seasonal precipitation i.e., before evaporation. Since the soil texture of Radkan is

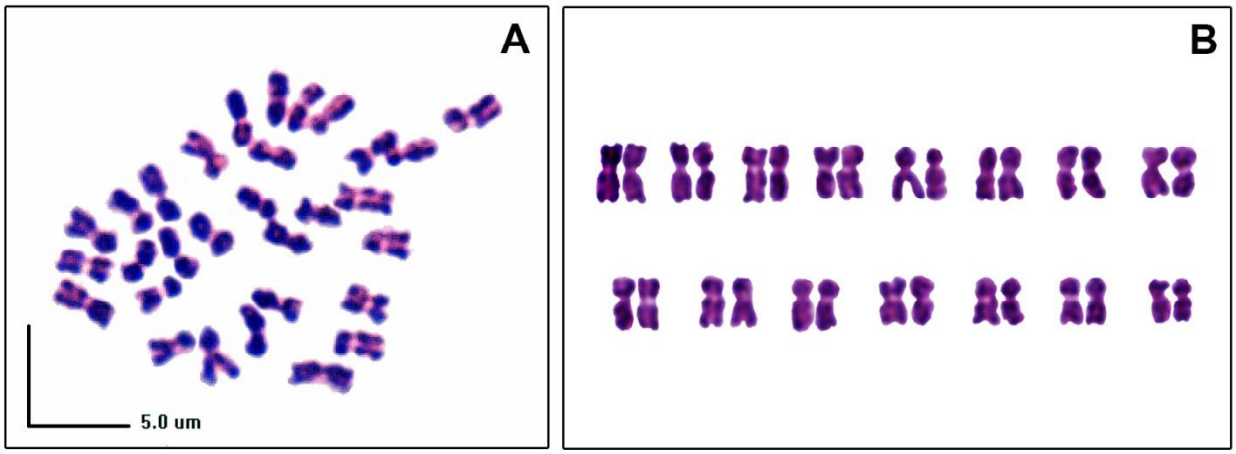


Fig. 6. The karyological characteristics in ***A. ejtehadii***. **A**. Metphasic cell. **B**. Karyotype.

clay-loam, it is susceptible to waterlogging, due to the weak infiltration, causing poor root aeration (Gill *et al.*, 2004). Roots of *A. ejtehadii* can escape the waterlogging conditions through growing near the soil surface, a mechanism that was also seen in marsh plants kilogram nitrogen and phosphorus, is considered as a poor soil in terms of the nutrient content (Esu, 1998;Kparmwang *et al.*, 2001). This soil is rich in potassium and calcium carbonate with 403 mg/kg and 38 percent, respectively (Rosen et al., 1998). The vegetation of Radkan region is relatively poor and it seems that the poor soil nutrient content in Radkan doesn't allow plants to thrive in this region. It was also reported that A. microphyllum can grow in the same soil conditions (Shokri *et al.*, 2003).

Acanthophyllum ejtehadii is a chamaephyte cushion plant. Different life forms of plant can be attributed to the adaptation to the climatic conditions of its habitats (Asaadi, 2009). This vegetative form of plants is a possible indicator of unfavorable climatic and poor soil conditions (Barik and Misra, 1998; Malik *et al.*, 2007).

Acanthophyllum ejtehadii grows in all slightly steep slope fields of Radkan region. It is also reported that A. microphyllum grows in clay loam soils with poor nutrient content and in all inclined fields (Shokri *et al.*, 2003).

Field observation revealed that A. ejtehadii expands its distribution range only by sexual reproduction. Maleki *et al.* (2015a) reported that the germination percentage of A. ejtehadii's small seeds were 100 by scarification treatment in Vitro. Wind-induced-dispersion of *A. ejtehadii*'s seeds may result in their scratching by surrounding bed rocks. This natural scarification facilitates the germination of seeds. This strategy helps *A. ejtehadii* to avoid intraspecific competition by inhibition of seed germination under maternal canopy (Maleki *et al.*, 2015a).

Regarding the shape and orientation of the bract and floral leaves, Mahmoudi *et al.* (2012) have considered (Atwell *et al.*, 1999). This mechanism isn't routine for all the *Acanthophyllum* species. For instance, A. pachystegium has a taproot with a little peripheral growth.

Radkan's soil, with its 0.5 and 6.53 grams per the A. ejtehadii similar to A. diezianum, and in terms of hairs similar to A. pachystegium. Based on the results of the current study branching pattern of buds and characteristics of hairs would be suitable distinguishing characters to identify *A. ejtehadii* from A. diezianum. The shape and orientation of the bracts and floral leaves are also proper differential properties to identify A. ejtehadii from both A. lilacinum and A. pachystegium.

There are extensive data on Caryophyllaceae anatomical characteristics. hair type (Davis, 1967), Calcium oxalate crystals in parenchyma (Schweingruber, 2007), and oval seeds with curved embryo (Schiman-Czeika, 1988; Ghahraman, 2004) are the notable features of *Acanthophyllum* sp. These specific characteristics are also observed in *A. ejtehadii*. Maleki *et al.* (2015 b) reported that this species has curved embryo, oval seeds, and pantopolyporate spherical pollen grains, which is a general characteristic in *Acanthophyllum* taxa (Mahmoudi-Shamsabad *et al.*, 2013).

Acanthophyllum ejtehadii is a diploid species and has homogenous karyotype with 12m+3sm formula. Ghaffari (2004) studied karyological features of 17 Acanthophyllum species, and reported that this taxon has x=14-15 basic chromosome numbers. Most species of Oligosperma and Macrostegia sections are diploid with $2n=2x=30$. However, *Acanthophyllum* and *Plesiosperma* sections are tetraploid ($2n=4x=60$) and hexaploid ($2n=6x=90$), respectively. It was also reported that polyploidy played an important role in speciation and evolution of this genus.

ACKNOWLEDGEMENTS

The authors express their appreciation to the Research Council of Ferdowsi University of Mashhad for the financial support of the research project (Project No. 3/30192). Moreover, the authors gratefully acknowledge Mr. M. Hassanzadeh for his constructive comments during the language edition of the paper. The authors would like to thank Dr. A. Pirani for her comments during the revision of this manuscript.

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