

[Research]

## A revision of chestnut-leaved oak (*Quercus castaneifolia* C. A. Mey.; *Fagaceae*) in Hyrcanian Forests of Iran

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### ABSTRACT

Chestnut-leaved oak (*Quercus castaneifolia* C. A. Mey) is one of the most important native oaks of Iran distributed in the Hyrcanian Forests. The pure and mixed stands of it cover about 6.5% of these forests. The species represents morphological variations which have resulted in the description of several infraspecific taxa by different authors. Eight taxa were recognized as subspecies and varieties of *Q. castaneifolia*. In this survey, a set of quantitative and qualitative micro-morphological characteristics such as type of trichome, number and length of trichome rays, type of epicuticular waxes, type and shape of stomata, shape and sculptural features of pollen exine were studied using light microscopy and scanning electron microscopy. These characters were analyzed by the discriminant analysis method and combined with the macromorphological characters to designate the variation within the species and to evaluate the existing infraspecific taxa. Based on our results, the studied taxa are defined into four infraspecific taxa as follows: *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia*, *Q. castaneifolia* subsp. *castaneifolia* var. *minuta*, *Q. castaneifolia* subsp. *aitchisoniana*, *Q. castaneifolia* subsp. *undulate*. The studied characteristics showed to be diagnostic for identification of the infraspecific taxa.

**Keywords:** Hyrcanian Forests, *Quercus castaneifolia*, Pollen, Trichome, SEM.

### INTRODUCTION

*Quercus* is the largest genus in the family *Fagaceae* with about 300 (Lawrence 1951) - 600 species (Soepadmo 1972). This genus includes evergreen and deciduous shrubs and trees extending from cold latitudes to tropical Asia and Americas. It is the most common genus of *Fagaceae* in forests of Iran (Sabeti 1994). Different species of oaks are distributed in vast areas (about 6 million ha) of Zagros, Arasbaran and Hyrcanian Forests. The genus shows a remarkable morphological variation in these forests.

*Quercus castaneifolia* is one of the most important species of Iran's native oaks, distributed in the Hyrcanian Forests. As a main species of the Hyrcanian forests, it forms two important forest communities in this zone: *Quercus-Buxetum* in the Caspian coast plains and *Quercus-Carpinetum* in the lowlands (up to 700m a.s.l). With drier climate in the northeast of Iran, the later

community gradually transforms into a *Zelkovo-Quercetum* community. *Quercus castaneifolia* is found in composition with other woody species on the upper altitudes. Pure and mixed stands of *Q. castaneifolia* cover about 6.5% of the Hyrcanian Forests (Forest, Range & Watershed Management of Iran, 2003).

This species shows morphological variations in leaf and acorn characteristics. The delimitation of *Q. castaneifolia*, number of its subspecies and varieties varies according to different authors. At first, Meyer (1831) described *Q. castaneifolia* C. A. Mey. from the Caucasus and Hyrcanian Forests of Iran. Then Schwarz (1935) studied the herbarium specimens collected by Bornmüller from Bandar-Gaz forests in Golestan Province, and Rasht forests in Guilan province. He described a new species namely *Q. sintenisiana* O. Schwarz, based on trichome characteristics, Three

years later, Camus (1936-1938) reported two subspecies including: *Q. castaneifolia* C. A. Mey. subsp. *eucastaneifolia* A. Camus and *Q. castaneifolia* C. A. Mey. sub sp. *aitchisoniana* A. Camus in his monograph of world's oaks. The most complete study of *Quercus* in Iran was done by Djavanichir Khoie (1967) based on leaf and acorn morphology. He classified the Iranian oaks into two subgenera; *Quercus* (lobed-leaved species) and *Complanate* (dentate-leaved species), based on leaf venation. The subgenus *Complanate* was further divided into two sections; *Oligandrae* Djav.-Khoie and *Polyandrae* Djav.-Khoie based on number of stamens. According to this classification *Q. castaneifolia* belongs to section *Oligandrae*. Djavanichir Khoie treated *Q. sintenisiana* O. Schwarz and *Q. castaneifolia* C. A. Mey. subsp. *eucastaneifolia* A. Camus as synonyms of the type species but he accepted *Q. castaneifolia* C. A. Mey. subsp. *aitchisoniana* A. Camus as a separate taxon and introduced 6 new taxa from the Hyrcanian Forests as follows:

- *Q. castaneifolia* C. A. Mey. subsp. *castaneifolia* var. *ellipsoidalis* Djav.-Khoie
- *Q. castaneifolia* C. A. Mey. subsp. *castaneifolia* var. *minuta* Djav.-Khoie
- *Q. castaneifolia* C. A. Mey. subsp. *incurvata* Djav.-Khoie
- *Q. castaneifolia* C. A. Mey. subsp. *subrotundata* Djav.-Khoie
- *Q. castaneifolia* C. A. Mey. subsp. *triangularis* Djav.-Khoie
- *Q. castaneifolia* C. A. Mey. subsp. *undulata* Djav.-Khoie

Menitsky in *Flora Iranica* (1971), categorized Iran's native oaks into subgenus: *Quercus*, and two sections: *Quercus* and *Cerris*. He placed dentate-leaved species in section *Cerris* and lobed-leaved species in section *Quercus*. According to his classification, *Q. castaneifolia* was considered in section *Cerris*, subsec. *Cerris* and all infraspecific taxa recognized by Djavanichir Khoie and Camus were treated as its synonyms. He recognized only *Q. castaneifolia* C. A. Mey. subsp. *castaneifolia* from Iran.

Leaf epidermal structures and pollen micromorphology have been often used for classification and identification of *Quercus* species and based on the previous researches, these characteristics are helpful

for taxonomic purposes (Monoszon 1962; Smit 1973; Hardin 1976, 1979a, 1979b; Thamsom & Mohlenbrock 1979; Bačić 1981; Colombo et al. 1983; Solomon 1983a, 1983b; Uzunova & Palamarev 1985, 1992a, 1992b, 1993; Jones 1986; Wang & Chang 1988; Safou & Saint-Martin 1989; Jarvis et al. 1992; Penas Merino et al. 1994; Llamas et al. 1995; Uzunova et al. 1997; Zheng et al. 1999; Kedves et al. 2002; Nikolić et al. 2003; Scareli-Santos et al. 2007).

In this research, we focused on the foliar epidermis structure and pollen micromorphology of *Q. castaneifolia* as well as, its subspecies and varieties, introduced by Djavanichir Khoie, to evaluate the taxonomic significance of these characteristics for the identification of the infraspecific taxa. A complete survey on habitats of these taxa, their distribution patterns, populations and ecological conditions were also carried out.

#### Materials and Methods

The majority of specimens used in this study were obtained during several field trips to areas according their geographic ranges in Hyrcanian Forests of the country. At least 10 trees were studied for each taxon. Furthermore other previously collected specimens of the Herbarium of Research Institute of Forests and Rangelands of Iran (TARI) and the Herbarium of Natural Resources Faculty, University of Tehran (NRF) were sampled. Materials and collected data of some individual plant samples of all studied taxa are listed in table 1. The voucher specimens are deposited in TARI.

Fully matured anthers and leaves were collected in spring and summer. Leaf samples were collected from open-grown canopy branches that showed limited morphological variations, resulting from environmental factors such as exposure and directions (Baranski 1975; Blue & Jensen 1988). The micromorphological features and quantitative characters of pollen grains were studied by Hitachi (S-4160) scanning electron microscope at an accelerating voltage of 15 KV and photographed by Olympus camera using a ×100 eyepiece. Pollen grains were prepared by the standard acetolysis method and then they were mounted on glycerin jelly and sealed with paraffin wax for LM (Harley 1992). Measurements were taken from 50 grains of

each examined taxon and the mean, range and standard deviation were calculated for polar axis (P) and equatorial diameter (E). The ratio of polar axis to equatorial diameter (P/E) was provided as an index of pollen shape (Faegri & Iversen 1964). The exine thickness of pollen grains was measured as well. For SEM observation, the pollen grains were mounted on aluminum stubs, air dried at room temperature and coated with gold. In the case of leaves, epidermal structures such as trichome types, number and length of the trichome rays, dimension and location of stomata and type of epicuticular waxes were studied by SEM. The leaf measurements were taken from trichomes and stomata, about midway between the base and apex of mid-vein and margins. Only fully expanded, undamaged leaves without signs of scarring or disease were examined. The leaf specimens were mounted on aluminum stubs and sputter-coated with

gold palladium for 5 minutes in a Humer II Sputtering Device. All leaf materials were prepared without pretreatment or critical point drying. Quantitative characters were calculated from 50 representative trichomes and stomata per examined taxon. The trichome ray length was measured from the point of divergence of the ray and at the base of the trichome. Terminology used for trichome types is based on Hardin (1976, 1979a). Identification of wax layer types is carried out according to Koch & Barthlott (2009). Terminology used for pollen shapes and features are based on Erdtman (1986), Punt et al. (1994) and Halbritter et al. (2006). Five quantitative variables of leaf and pollen including number and length of the trichome rays, length/width ratio of stomata (as shape indicator), polar length and equatorial diameter of pollen grains were analyzed by the discriminant analysis method to differentiate the examined taxa.

**Table 1.** List of taxa and the collection data

Taxon	Collection data
<i>Q. castaneifolia</i> subsp. <i>castaneifolia</i> var. <i>castaneifolia</i>	Iran: Golestan, Gorgan, Tuskaestan, 850m, Panahi and Pourhashemi, 95097 (TARI) Iran: Guilan, Astara, Khanehaye-Asiab village, 63m, Panahi and Pourhashemi, 95078 (TARI) Iran: Tehran, Cultivated in National Botanical Garden of Iran, 1320m, Panahi 95084 (TARI)
- - - - var. <i>ellipsoidalis</i>	Iran: Guilan, Astara, Sheikh mahalleh village, 25m, Panahi and Pourhashemi, 95074 (TARI) Iran: Mazandaran, Chalus, Dashte nazir,, 945m, Panahi and Pourhashemi, 95754 (TARI) Iran: Tehran, Cultivated in National Botanical Garden of Iran, 1320m, Panahi, 95089 (TARI)
- - - - var. <i>minuta</i>	Iran: Mazandaran, Zirab, Lajim village, 890m, Panahi and Pourhashemi, 95100 (TARI) Iran: Mazandaran, Zirab, Lajim village, 890m, Panahi and Pourhashemi, 95060 (TARI) Iran: Mazandaran, Zirab, Lajim village, 950m, Djavan chir, 2122 (NRF)
<i>Q. castaneifolia</i> subsp. <i>aitchisoniana</i>	Iran: Golestan, Gorgan, Tuskaestan, 1800m, Panahi and Pourhashemi, 95001(TARI) Iran: Semnan, Foulad Mahalleh, Rudbarak village, 2031m, Panahi and Pourhashemi, 95006 (TARI)
<i>Q. castaneifolia</i> subsp. <i>incurvata</i>	Iran: Mazandaran, Chalus, Dashte nazir, 945m, Panahi and Pourhashemi, 95082 (TARI) Iran: Mazandaran, Chalus, Dashte nazir, Gat-Kesh village, 1180m, Panahi and Pourhashemi, 95752 (TARI)
<i>Q. castaneifolia</i> subsp. <i>subrotundata</i>	Iran: Guilan, Astara, Khanehaye - Asiab village, 76m, Panahi and Pourhashemi, 95079 (TARI) Iran: Ardabil, Fandoghlu Forest, 1442m, Panahi and Pourhashemi, 95080 (TARI)
<i>Q. castaneifolia</i> subsp. <i>triangularis</i>	Iran: Mazandaran, Chalus, Veisar, 1400m, Djavan chir, 2230 (NRF) Iran: Ardabil, Fandoghlu Forest, 1442m, Panahi and Pourhashemi, 95101 (TARI) Iran: Mazandaran, Chalus, Dashte nazir, Gat-Kesh village, 1220m, Panahi and Pourhashemi, 95753 (TARI)
<i>Q. castaneifolia</i> subsp. <i>undulata</i>	Iran: Guilan, Astara, Asgar mahalleh village, 10m, Panahi and Pourhashemi, 95077 (TARI) Iran: Guilan, Astara, Asgar mahalleh village, 20m, Panahi and Pourhashemi, 95755 (TARI)

## RESULTS

### Discriminant analysis of studied taxa

The summary of canonical discriminant analysis is reported in table 2-5. Five canonical functions are separated, so that the first two functions describe 75.5% of variance. Standardized coefficients of variables showed that the most discriminative variable is the length of trichome rays. Based on studied variables, there are significant differences between the 8 studied taxa. As it could be seen in canonical graph (Fig. 1), some taxa set into near each other which mean high similarity between them. After combination of discriminate analysis results with leaf and

acorn morphological characters and qualitative characters of their pollen grains, all of studied taxa are classified into four distinct new groups as stated below and other taxa treated as synonyms with them.

- Group 1: *Quercus castaneifolia* subsp. *castaneifolia* var. *castaneifolia*,

*Q. castaneifolia* subsp. *castaneifolia* var. *ellipsoidalis* and *Q. castaneifolia* subsp. *subrotundata*

- Group 2: *Quercus castaneifolia* subsp. *castaneifolia* var. *minuta*

- Group 3: *Quercus castaneifolia* subsp. *aitchisoniana*, *Q. castaneifolia* subsp. *triangularis* and *Q. castaneifolia* subsp. *incuroata*

- Group 4: *Quercus castaneifolia* subsp. *undulata*

The misclassification value is 4.7%.

**Table 2.** Tests of equality of group means in discriminant analysis

Variable	Walk's Lambda	F	df1	df2	Sig.
Number of trichome rays	0.541	47.432	7	392	0.000
Length of trichome rays	0.443	70.524	7	392	0.000
Length/width ratio of stomata	0.504	55.090	7	392	0.000
Polar length of pollen	0.574	41.563	7	392	0.000
Equatorial diameter of pollen	0.632	32.540	7	392	0.000

**Table 3.** Eigenvalues of functions in discriminant analysis

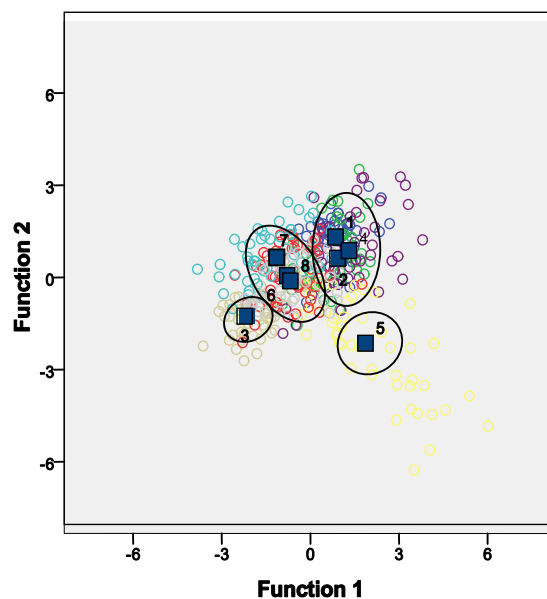
Function	Eigenvalue	% of Variance	Cumulative (%)	Canonical correlation
1	2.296	50.2	50.2	0.835
2	1.160	25.3	75.5	0.733
3	0.816	17.8	93.4	0.670
4	0.293	6.4	99.8	0.476
5	0.010	0.2	100	0.098

**Table 4.** Wilk's Lambda values of functions in discriminant analysis

Test of function(s)	Wilk's Lambda	Chi-square	df	Sig.
1 through 2	0.059	1109.420	35	0.000
2 through 5	0.195	641.241	24	0.000
3 through 5	0.422	339.012	15	0.000
4 through 5	0.766	104.760	8	0.000
5	0.990	3.764	3	0.288

**Table 5.** Standardized canonical discriminant function coefficients

Variable	Function				
	1	2	3	4	5
Number of trichome rays	0.182	0.396	0.877	-0.167	0.146
Length of trichome rays	0.681	-0.841	0.205	0.612	-0.175
Length/width ratio of stomata	0.178	0.301	-0.310	0.406	-0.091
Polar length of pollen	0.564	0.100	-0.151	-0.616	-0.525
Equatorial diameter of pollen	0.493	0.020	-0.347	-0.197	0.779



**Fig. 1. Canonical discriminant functions of studied taxa:** (1) *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia* (2) *Q. castaneifolia* subsp. *castaneifolia* var. *ellipsoidalis* (3) *Q. castaneifolia* subsp. *castaneifolia* var. *minuta* (4) *Q. castaneifolia* subsp. *subrotundata* (5) *Q. castaneifolia* subsp. *undulate* (6) *Q. castaneifolia* subsp. *triangularis* (7) *Q. castaneifolia* subsp. *aitchisoniana* (8) *Q. castaneifolia* subsp. *incurvata*

#### Description of observed trichomes

Both leaf abaxial and adaxial surfaces of the studied taxa are covered with trichomes, but trichomes density and variability are higher on the abaxial surface. We have identified five different trichome types as follows:

1. Simple-uniseriate type: Thin-walled, unicellular-multicellular and uniseriate trichome with different length, diameter, cell number and shape. This type of trichome that was observed only on the abaxial surface of all studied taxa is multicellular (e.g. Fig. 2F).

2. Solitary type: Single, long, usually straight and unicellular often thin-walled. This type is composed of two very distinct subtypes. The first one comprises an erect, long, unicellular hair on the lamina and veins. The second subtype includes more or less appressed, long, often thin-walled, sometimes crispate hairs (Figs. 2O and 2M), mostly on juvenile leaves and may persist along the petiole and midrib. This type was observed often on the midrib of all studied taxa (e.g. Figs. 2E, 2H).

3. Fasciculate type: Erect, thick-walled cells, clustered and fused at the base, with number of rays varying from 2-12 in a single set. This type of trichome may be sessile, stipitate or pedicled. We have found 2-rays sessile-fasciculate trichomes on the adaxial surface (e.g. *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia*, Fig. 2D) and stipitate-fasciculate with more rays on the midrib (e.g. *Q. castaneifolia* subsp. *castaneifolia* var. *minuta*, Fig. 2H).

4. Multiradiate type: Thick-walled, clustered or tufted trichome with rays radiating from more than one level including: the outer erect or more or less horizontal, and the inner erect ones. This trichome type was observed in *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia* (Fig. 2B).

5. Stellate type: Usually thick-walled trichome, with a single set of radiating, slender rays, projecting horizontally from a common center, sessile or stipitate. Stellate trichome was the most abundant type in all examined taxa. We found both of sessile (e.g. *Q. castaneifolia* subsp. *aitchisoniana*,

Fig. 2I) and stipitate (e.g. *Q. castaneifolia* subsp. *undulata*, Fig. 2M) trichomes in examined taxa. The number of rays in stellate trichomes changes, from 3-4 in *Q. castaneifolia* subsp. *castaneifolia* var. *minuta* (Fig. 2F) to 8-18 in *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia* (Fig. 2A). The ray length of this trichome varies greatly, from 50-150  $\mu\text{m}$  in *Q. castaneifolia* subsp. *castaneifolia* var. *minuta* (Fig. 2F) to 100-450  $\mu\text{m}$  in *Q. castaneifolia* subsp. *undulata* (Fig. 2L). The differences in trichome types on the abaxial and adaxial surfaces of different taxa are summarized in table 6.

#### Description of observed waxes

Only one type of epicuticular waxes, with smooth layer, was recognized on the abaxial and adaxial surfaces of all studied taxa. Crust type is a type of epicuticular waxes, superimposed continuously with a thickness of usually less than 1  $\mu\text{m}$  on the cuticle without a prominent surface sculpturing (Figs. 3A, 3B).

#### Description of observed stomata

We identified an elliptical shape of stomata in all examined taxa except in *Q. castaneifolia* subsp. *castaneifolia* var. *minuta* with round stomata (Fig. 3D-F). Stomata were raised above the epidermal surface in all cases. The rims of stomata were entirely covered by epicuticular waxes of smooth layer type in all studied taxa, but the pore was visible. Quantitative features of

trichomes and stomata of the examined taxa are presented in table 7.

#### Description of observed pollen

The morphology of pollen grains is similar among the studied taxa as follows: monad pollen, tricolpate or tricolporate; with medium length colpi (Fig. 4I), pores sometimes indistinct, geniculus sometimes present; oblate-spheroidal shape. The pollen grains observed in this study are similar in size. They are isopolar (Figs. 4A-C). In most pollen grains, the polar axis is shorter than the equatorial diameter and in some pollen grains the polar axis and the equatorial diameter are almost equal in length. The length of polar axis varies from 23-34  $\mu\text{m}$ . The length of equatorial diameter ranges from 26-40  $\mu\text{m}$ . The P/E ratio and the exine thickness vary from 0.90-0.94  $\mu\text{m}$ , and 1-2  $\mu\text{m}$ , respectively. The exine thickness is equal in mesocolpium and apocolpium. The pollen size of the examined taxa is categorized in medium class (26-50  $\mu\text{m}$ ) (Figs. 4J-M). Exine ornamentation in apocolpium and mesocolpium is mostly similar, except in *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia*.

The pollen grain sculpturing is composed of two tiers: the first basal tier (at the tectum surface) and the second tier including superimposed projections covering the first basal tier.

**Table 6.** Trichome types of studied taxa

Taxon	Trichome types				
	Simple-uniseriate	Solitary	Fasciculate	Multiradiate	Stellate
<i>Q. castaneifolia</i> subsp. <i>castaneifolia</i> var. <i>castaneifolia</i>	-/+	-/+	+/+	-/+	+/+
- - - - - var. <i>minuta</i>	-/+	+/+	-/+	-/-	+/+
<i>Q. castaneifolia</i> subsp. <i>aitchisoniana</i>	-/+	-/+	-/+	-/-	+/+
<i>Q. castaneifolia</i> subsp. <i>undulata</i>	-/+	-/+	-/+	-/-	+/+

Adaxial surface/ Abaxial surface. - = absent, + = present

**Table 7.** Quantitative features of stellate trichomes and stomata of studied taxa

Taxon	Number of rays		Length of rays		Stomata		
	Range	Mean $\pm$ SD	Range ( $\mu\text{m}$ )	Mean ( $\mu\text{m}$ ) $\pm$ SD	Length ( $\mu\text{m}$ ) Mean $\pm$ SD	Width ( $\mu\text{m}$ ) Mean $\pm$ SD	Ratio
<i>Q. castaneifolia</i> subsp. <i>castaneifolia</i> var. <i>castaneifolia</i>	4-18	9.2 $\pm$ 0.43	84-180	120.3 $\pm$ 19	19.5 $\pm$ 1.5	12.6 $\pm$ 0.5	1.56
- - - - - var. <i>minuta</i>	2-8	4.3 $\pm$ 0.21	52-136	95.1 $\pm$ 20.1	17.7 $\pm$ 2.2	16.5 $\pm$ 1.5	1.19
<i>Q. castaneifolia</i> subsp. <i>aitchisoniana</i>	4-10	6.2 $\pm$ 0.33	51-170	116.6 $\pm$ 28.9	21.2 $\pm$ 2	12.6 $\pm$ 0.8	1.66
<i>Q. castaneifolia</i> subsp. <i>undulata</i>	4-10	7.8 $\pm$ 0.36	104-450	259.4 $\pm$ 113.1	21.1 $\pm$ 0.7	16.6 $\pm$ 1.6	1.28

In all the studied taxa, the first basal tier is scabrate-rugulate with perforation and the second tiers includes micro-scabrate, micro-striate and micro-psilate. Pore density (dense and sparse pore), and

distribution are variable. The main quantitative features of the polar length and the equatorial width of pollen are presented in table 8.

**Table 8.** Minimum, Mean, Maximum values and standard deviation of the polar length and equatorial width of pollen

Taxon	Polar length ( $\mu\text{m}$ )	Equatorial width ( $\mu\text{m}$ )	Ratio
<i>Q. castaneifolia</i> subsp. <i>castaneifolia</i> var. <i>castaneifolia</i>	27 (30) $33 \pm 1.4$	29 (32) $34 \pm 1.5$	0.94
- - - - var. <i>minuta</i>	24 (27) $32 \pm 1.6$	26 (30) $33 \pm 1.8$	0.93
<i>Q. castaneifolia</i> subsp. <i>aitchisoniana</i>	23 (27) $34 \pm 2.6$	26 (31) $40 \pm 3.2$	0.90
<i>Q. castaneifolia</i> subsp. <i>undulata</i>	26 (30) $34 \pm 1.5$	30 (33) $36 \pm 1.7$	0.91

### Description of taxa

A summarized description of the examined taxa is presented below.

***Quercus castaneifolia*** C. A. Mey. subsp. *castaneifolia* var. *castaneifolia*, Verzeichn. d. Pflanz. Cauc., 44 (1831).

Syn.: *Q. castaneifolia* C. A. Mey. subsp. *castaneifolia* var. *ellipsoidalis* Djav.-Khoie, Chenes Iran, 68 (1967); *Q. castaneifolia* C. A. Mey. subsp. *subrotundata* Djav.-Khoie, Chenes Iran, 73 (1967).

Deciduous large tree, up to 40m high, with expanded crown, dark gray thick bark and caduceus leaves. It is distributed in Caucasus and throughout the Hyrcanian Forests of Iran. It has been observed from sea level up to an altitude of 2400m in these forests. The cupule scales are tough, tomentose, gray color and reflexed. The scale length gradually increases up to 6 mm at the base of cupule. The most frequent trichomes on the abaxial surface are sessile-stellate and multiradiate types. These trichomes have 4-18 (often 8-14) rays measuring 80-180  $\mu\text{m}$ . The inner rays of multiradiate trichomes are usually erect and the outer ones are more or less horizontal, usually appressed on the lamina (Fig. 2B). The multicellular simple-uniseriate hairs are present on the abaxial surface. The trichomes of the adaxial surface are restricted only to a few stellate ones with 3-10 (often 4-6) rays and 2-rayed fasciculate forms, irregularly and sparsely scattered on the epidermal surface (Fig. 2D). The midrib is covered by solitary, medium length trichomes, long fasciculate trichomes with 2-6 erect rays and 4-rayed stellate trichomes (Fig. 2E). The stellate

trichomes may be more prostrate with the rays thicker than usual. The stomata are raised. Both the stomata and the rim are clearly elliptical in shape (Fig 3C). The sculpturing pattern of the pollen at the first tier is rugulate with sparse pores and irregularly arranged ornamentation elements. The sculpturing pattern in the second tier is striate-rugulate with micro-psilate suprasculpture (Figs. 4D-E).

***Quercus castaneifolia*** C. A. Mey. subsp. *castaneifolia* var. *minuta* Djav.-Khoi, Chenes Iran, 69 (1967).

Deciduous large tree, up to 25m high, with expanded crown, dark gray thick bark and caduceus leaves. Distribution of this variety is restricted to Lajim and Veisar forests, in Mazandaran Province and the Gorgan forests, in Golestan Province. The acorn is characterized by a completely convex hilum and a black-brown ring around it. In this taxon the size of the gland is smaller than that of the type variety. The trichome density is less than that of type variety; therefore the stomata are clearly visible. The most abundant trichomes on the abaxial surface are sessile-stellate type with 2-8 (often 3-4) rays and 50-140  $\mu\text{m}$  long and completely appressed on the lamina. A few solitary and simple-uniseriate hairs are present on the abaxial surface. Trichomes are restricted only to a few solitary and stellate hairs with 2-4 rays, irregularly and sparsely scattered on the epidermal surface of adaxial surface (Fig. 2G). On the midrib, solitary, long fasciculate with 2-4 erect rays

and 4-rayed stellate trichomes were found (Fig. 2H). The stomata are raised, round in shape and their dimensions are smaller than that of type species (Fig. 3D). The sculpturing pattern of the pollen at the first tier is scabrate-rugulate with sparse pore. The ornamentation of the second tier is striate-rugulate with suprasculpture micro-psilate to micro-scabrate (Fig. 4F).

*Quercus castaneifolia* C. A. Mey. subsp. *aitchisoniana* A. Camus, Monogr. Gen. *Quercus* 1: 557 (1936-1938).

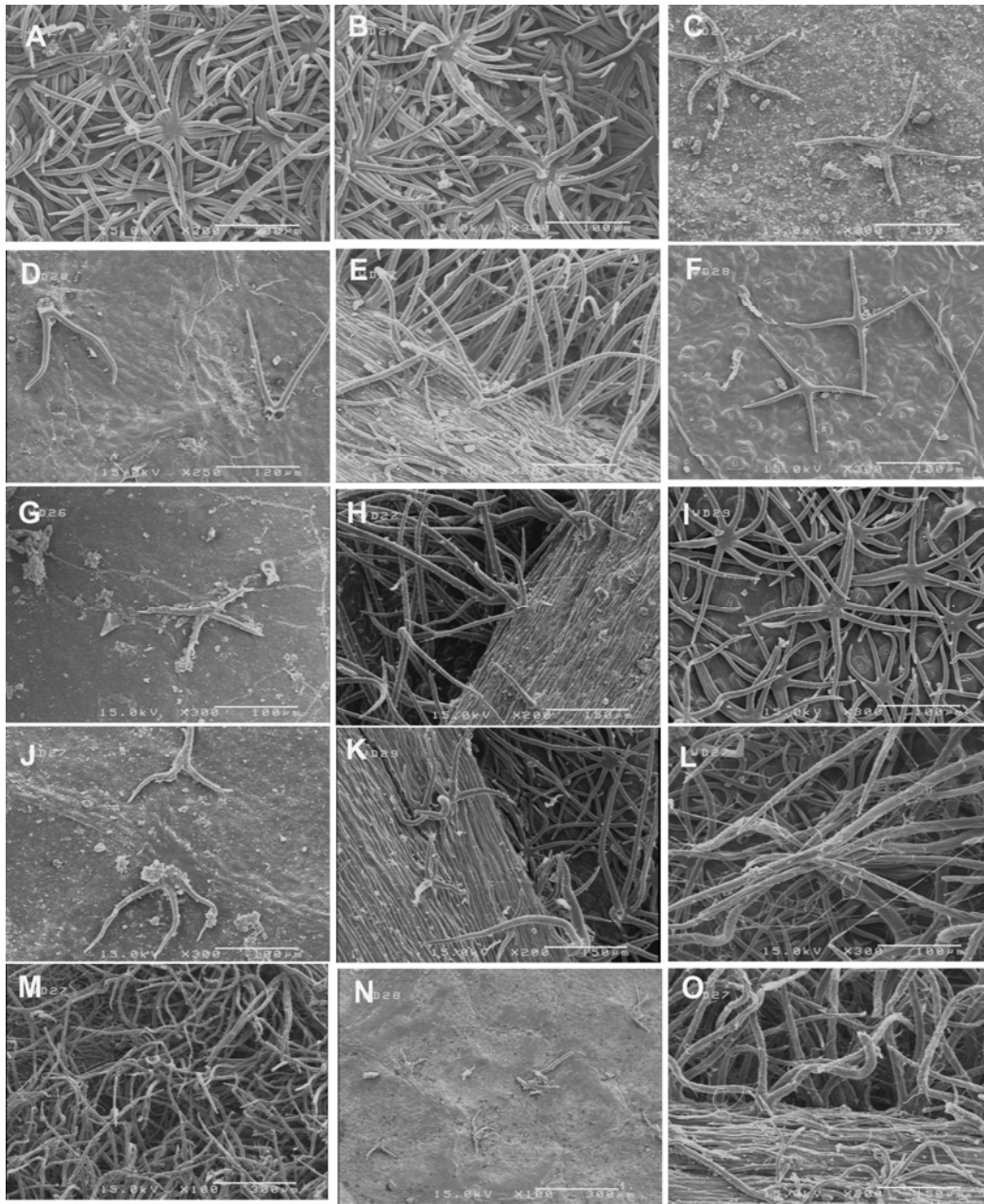
Syn.: *Q. castaneifolia* C. A. Mey. subsp. *triangularis* Djav.-Khoi, Chenes Iran, 75 (1967); *Q. castaneifolia* C. A. Mey. subsp. *incurvata* Djav.-Khoi, Chenes Iran, 72 (1967).

Deciduous medium size tree, up to 15m high, with expanded crown, dark gray thick bark and caduceus leaves. Its distribution is restricted only to middle and upper altitudes of the Hyrcanian Forests. The size of acorn is smaller than that of the type variety. The cupule is semi-spherical with completely erect, triangle shape scales that connect to cupule without any curving. Sessile-stellate trichomes with 4-10 (mostly 4-8) rays 50-170  $\mu\text{m}$  long, completely appressed on the lamina are the most frequent type found on the abaxial surface. The density of the stellate trichomes is almost identical to that of the type variety, but the number of rays is less than that of the type variety. A few simple-uniseriate hairs are present on the abaxial surface. There are only stellate trichomes with 2-4 rays on the adaxial surface and sparsely scattered trichomes on the epidermal surface. Long solitary, fasciculate with 2-6 erect rays (mostly 2) and stellate trichomes with 2-4 rays were observed on the midrib (Fig. 2K). The stomata are visible and raised. There are different sizes of elliptical stomata (Fig. 3E). The pollen sculpture pattern in the first tier is scabrate-rugulate with sparse

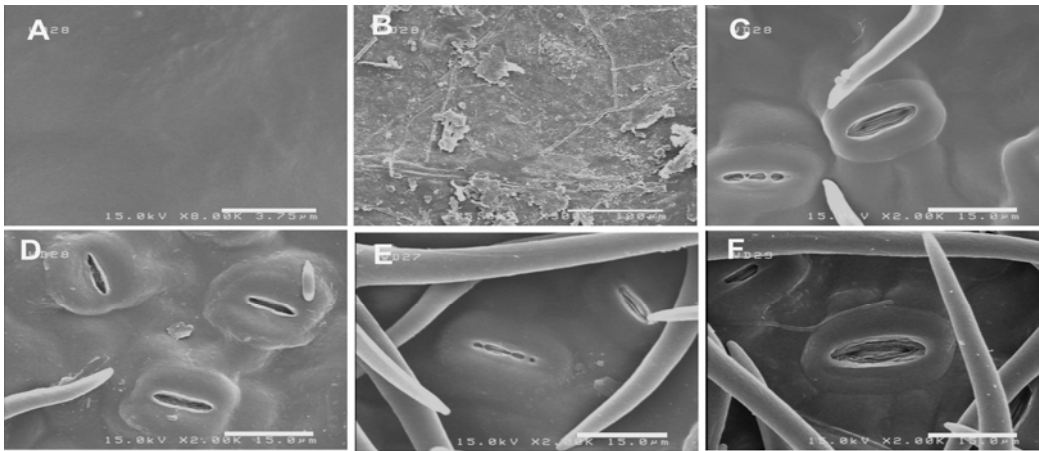
perforation. The second tier of exine sculpture is rugulate-scabrate and with micro-scabrate to micro-striate suprasculpture (Figs. 4G).

*Quercus castaneifolia* C. A. Mey. subsp. *undulate* Djav.-Khoi, Chenes Iran, 77 (1967). Deciduous large tree, up to 30m high, with expanded crown, dark gray thick bark and caduceus leaves. Its distribution is only restricted to bottomlands of Astara, in Guilan province. This taxon is characterized with big acorns, semi-spherical cupule and cylindrical gland. The cupule scales are thick, long (up to 13 mm) and reflexed. Two forms of stellate trichomes are found on the abaxial surface in different layers. Sessile-stellate trichomes with medium rays completely appressed on the lamina and stipitate-stellate trichomes with very long rays (100-450  $\mu\text{m}$ ) in the upper layer covered the sessile-stellate trichomes (Fig. 2L). In the second type of trichome, the rays are fused into an erect stipe and then diverge horizontally. The stellate trichomes have 4-10 (mostly 8) rays. Because of long rays, the stomata could hardly be observed. A few simple-uniseriate hairs are present on the abaxial surface. There are only stellate trichomes with 2-4 rays on the adaxial surface and sparsely scattered hairs on the epidermal surface. On the midrib, long solitary, fasciculate with 2-6 erect rays and stellate trichomes with 2-4 rays were observed (Fig. 2O). The stomata are raised (Fig. 2F). The first tier of sculpture pattern of pollen grain is psilate-scabrate with dens perforation (some of pores are lineate). While the second tier of sculpture is rugulate-scabrate. The size and shape of second tier features are irregular. The pores have different size and are regularly distributed. The suprasculpture is micro-scabrate (Fig. 4H).

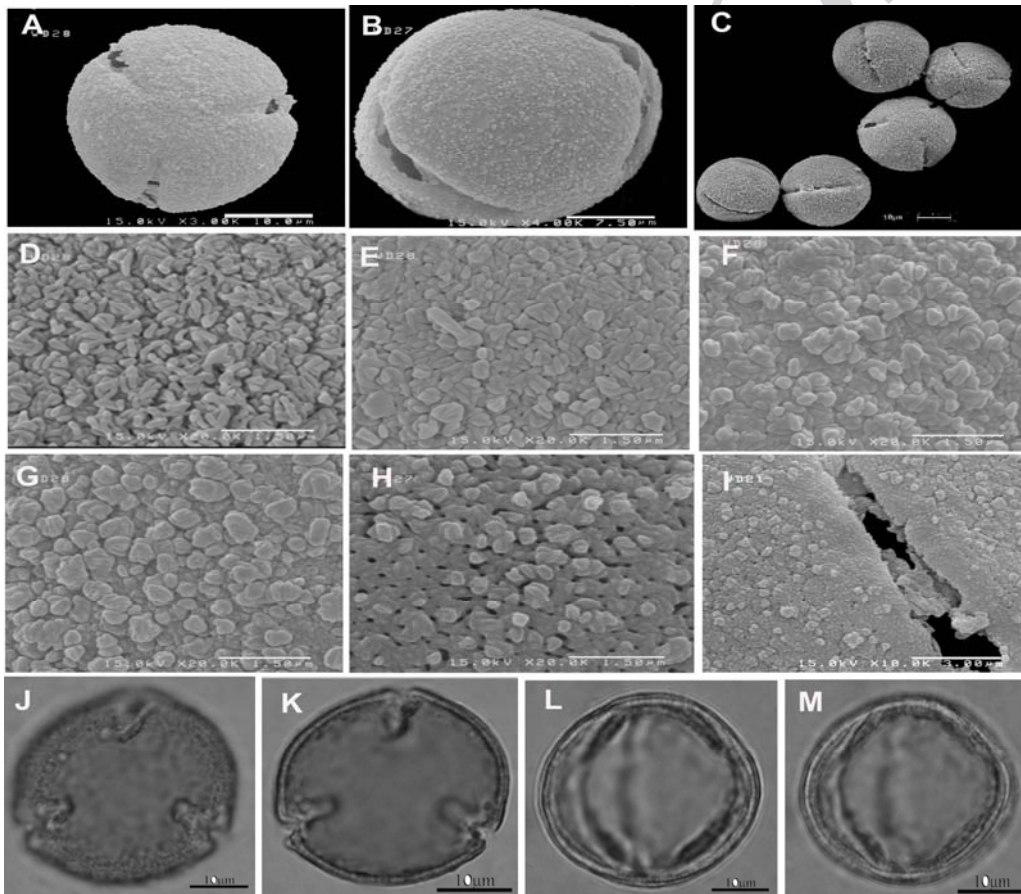




**Fig. 2. (A-O) SEM micrographs of trichomes and midrib in the subspecies and varieties of *Quercus castaneifolia*:** (A-E) *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia*, (F-H) *Q. castaneifolia* subsp. *castaneifolia* var. *minuta*, (I-K) *Q. castaneifolia* subsp. *aitchisoniana*, (L-O) *Q. castaneifolia* subsp. *undulate*. (A-B, E-F, H-I, K-M, O) Abaxial surface; (C-D, G, J, N) Adaxial surface, (A-C, F-G, I-J, L) scale bar = 100  $\mu$ m; (D) scale bar = 120  $\mu$ m; (E, H, K, O) scale bar = 150  $\mu$ m; (M-N) scale bar = 300  $\mu$ m



**Fig. 3.** SEM micrographs of wax and stomata in subspecies and varieties of *Quercus castaneifolia*: (A) wax on Abaxial surface, (B) wax on Adaxial surface, (C-F) Stomata, (C) *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia*, (D) *Q. castaneifolia* subsp. *castaneifolia* var. *minuta*, (E) *Q. castaneifolia* subsp. *aitchisoniana*, (F) *Q. castaneifolia* subsp. *undulate*, (A) scale bar = 3.75  $\mu$ m; (B) scale bar = 100  $\mu$ m; (C-F) scale bar = 15  $\mu$ m



**Fig. 4.** SEM & LM micrographs of pollen grains in subspecies and varieties of *Quercus castaneifolia*: (A) polar view, (B) equatorial view, (C) group of pollen, (D-E) mesocolpium and apocolpium, *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia*, (F) mesocolpium, *Q. castaneifolia* subsp. *castaneifolia* var. *minuta*, (G) mesocolpium, *Q. castaneifolia* subsp. *aitchisoniana*, (H) mesocolpium, *Q. castaneifolia* subsp. *undulate*, (I) colpus membrane, *Q. castaneifolia* subsp. *castaneifolia* var. *minuta*, (J-M) LM micrographs of pollen grains in *Q. castaneifolia*: (J-k) polar view, (J) high focus, (K) low focus, (L-M) equatorial view, (L) low focus, (M) high mid focus, (A, C, J-M) scale bar = 10  $\mu$ m; (B) scale bar = 7.5  $\mu$ m; (D-H) scale bar = 1.5  $\mu$ m; (I) scale bar = 3  $\mu$ m

## DISCUSSION

The taxonomy of oak species is very difficult (Muller 1952; Burger 1974; Hedge & Yaltirik 1982; Aas 1998) and besides the common morphological characters, other characteristic features are needed to elucidate the status of taxa and their variation. Micromorphological characteristics of leaf and pollen are useful in taxonomic treatment of oak species. In general, most of the authors (Boissier 1879; Parsa 1949; Menitsky 1971; Sabeti 1994) disagreed with specific nomenclature of species, varieties and forms of Iran's oaks. In this research we studied pollen and leaf characters of eight infraspecific taxa of *Q. castaneifolia*, using LM and SEM, which provided valuable data that could be inferred and used for identification of species.

Sabeti (1994) reported that the differences between subspecies and varieties of *Q. castaneifolia* are due to heterophyly, but according to Djavanichir Khoie (1967) the acorn shape and tree form are the basic characteristics for differentiating the subspecies and varieties of *Q. castaneifolia* in the north of Iran. As mentioned before, Djavanichir Khoie (1967) identified eight taxa within *Q. castaneifolia* in these forests. Some micromorphological characters of leaf and pollen such as type of trichome, number of trichome rays and ornamental features of pollen have proved to be diagnostic for separating the *Quercus* species. During last decades, these characters have been used widely throughout the world by botanists (e.g. Smit 1973; Hardin 1976, 1979a, 1979b; Solomon 1983a, 1983b; Safou & Saint-Martin 1989; Jarvis et al. 1992; Llamas et al. 1995; Nikolić et al. 2003; Liu et al. 2007).

In the current research, we have presented a detailed study on *Q. castaneifolia* complex from Iran using micromorphological characters of leaf and pollen.

Based on discriminant analysis, eight studied taxa are summarized into four taxa groups as follows:

Group 1: This group includes *Q. castaneifolia* subsp. *castaneifolia* var. *castaneifolia*, *Q. castaneifolia* subsp. *castaneifolia* var. *ellipsoidalis* and *Q. castaneifolia* subsp. *subrotundata*. The taxa belonging to this group are distributed throughout the Hyrcanian Forests from the Hyrcanian coast plains up to an altitude of

2400m. The reflexed scales of cupule are the obvious morphological characteristics in this group. The shape of gland has partial differences; long fusiform in type specimen, long cylindrical in *Q. castaneifolia* subsp. *castaneifolia* var. *ellipsoidalis* and smaller globose in *Q. castaneifolia* subsp. *subrotundata*. The taxa of this group have common micromorphological characteristics of leaf and pollen, such as multiradiate trichomes (not seen in other groups), the higher number of trichome rays (4-18) and the rugulate ornamentation on the exine surface. Group 2: This group consists of only *Q. castaneifolia* subsp. *castaneifolia* var. *minuta*, restricted to some regions of Lajim and Veisar forests in Mazandaran province and Gorgan forests in Golestan province. The black-brown ring around the hilum of gland is the best discriminative morphological characteristic to distinguish this taxon. Some micromorphological features such as limited number of trichome rays (often 3-4), short rays, lax trichomes, round stomata, scabrate-rugulate of first tire of sculpturing pattern and suprasculpture of micro-psilate to micro-scabrate are characteristic features of this taxon.

Group 3: This group includes *Q. castaneifolia* subsp. *triangularis*, *Q. castaneifolia* subsp. *aitchisoniana* and *Q. castaneifolia* subsp. *incurvata*, which are often distributed in middle to upper lands of the Hyrcanian Forests. From the morphological point of view, the cupules are semi-spherical with completely erect (not reflexed), triangle shape scales. This group has smaller acorn than those of other groups. The taxa of this group are clearly distinguished from the first group by having less number of trichome rays (often 4-8) and absence of multiradiate type of trichome. Pollen exine suprasculpturing is micro-scabrate to microstriate. The superimposed projections in this group are higher than those of other groups.

Group 4: *Q. castaneifolia* subsp. *undulata* is placed in this group. This taxon differs completely from other taxa by its acorn morphology. The acorn is big and cupule scales are very long (up to 13 mm) in this group. The acorn shape of this subspecies is similar to *Q. cerris*, the allied species of *Q. castaneifolia*. Presence of very long

trichome rays (up to 450  $\mu\text{m}$ ) are the diagnostic micromorphological character of this group. The pollen sculpturing is psilate to scabrate with dens perforations. So far, all of taxonomic studies on *Q. castaneifolia* in Iran have been done based on leaf and acorn morphology. Our study with emphasis on micromorphological characters of leaf and pollen confirmed 4 taxa of *Q. castaneifolia* in north forests of Iran. The results of previous research of Bussotti and Grossoni (1997) on micromorphological characters of the abaxial leaf surface of *Q. castaneifolia*, were identical to our results.

The results show that the leaf indumentum and pollen micromorphological characters are valuable features for assessing infraspecific classification of *Q. castaneifolia* in the Hyrcanian Forests of Iran.

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## مرور تاکسونومیکي بلندمازو در جنگل های هیرکانی

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### چکیده

بلندمازو یکی از مهمترین گونه های بلوط بومی جنگل های هیرکانی ایران است. توده های خالص و آمیخته این گونه حدود ۶/۵ درصد جنگل های هیرکانی را پوشانده اند. این گونه تغییرات مورفولوژیکی زیادی از خود نشان می دهد که منجر به توصیف چندین زیرگونه و واریته از آن توسط برخی گیاه شناسان شده است. در مجموع ۸ زیرگونه و واریته از بلندمازو شناسایی شده است. در این پژوهش مجموعه ای از ویژگی های کمی و کیفی ریزریخت شناسی برگ (از قبیل نوع کرک، تعداد و طول بازوهای کرک، نوع موم سطح برگ، نوع و شکل روزنه) و دانه گرده (ویژگی های ساختاری و تزئیناتی) با استفاده از میکروسکوپ نوری و الکترونی مطالعه شدند. ویژگی های مذکور با استفاده از روش آماری تحلیل ممیزی، تجزیه و تحلیل شدند و پس از تلفیق با ویژگی های ریخت شناسی، برای تفکیک زیرگونه ها و واریته های بلندمازو مورد استفاده قرار گرفتند. براساس نتایج بدست آمده تاکسون های مورد مطالعه در ۴ گروه طبقه بندی شدند که عبارتند از: *Quercus castaneifolia* subsp. *castaneifolia* var. *castaneifolia*، *Quercus castaneifolia* subsp. *castaneifolia* var. *minuta*، *Quercus castaneifolia* subsp. *undulata* و *Quercus aitchisoniana*. نتایج این تحقیق نشان داد که ویژگی های ریزریخت شناسی مورد استفاده از ارزش تاکسونومیکي حائز اهمیتی برخوردارند.