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MORPHOLOGICAL VARIATION OF FAGUS ORIENTALIS LIPSKY IN THE HYRCANIAN FORESTS OF IRAN

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Fagus orientalis Lipsky is the most important tree species of the Hyrcanian Forests of Iran. For long time, forest residents throughout Hyrcanian Forests have traditionally recognized *F. orientalis*, considering the appearance (outlook) of trees, into two forms: black beech, and white beech. In this research, we tried to evaluate micro-morphological characteristics of leaf and pollen grains of black and white beeches in the Hyrcanian Forests. Our research showed that there are no significant differences in micro-morphological characters between white and black beeches.

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Key words: *Fagus orientalis*; Hyrcanian Forests; leaf; micro-morphology; pollen

تغییرات ریختی راش شرقی (Fagus orientalis Lipsky) در جنگل‌های هیرکانی ایران

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راش شرقی (Fagus orientalis Lipsky) مهمترین گونه درختی جنگل‌های هیرکانی ایران است. جنگل‌نشینان این منطقه به‌طور سنتی با توجه به شکل ظاهری درختان راش، دو نوع راش به‌نام‌های راش سفید و راش سیاه را تفکیک می‌کنند. در پژوهش پیش‌رو سعی شد ویژگی‌های ریزریخت‌شناسی برگ و دانه گرده راش سفید و راش سیاه در جنگل‌های هیرکانی ارزیابی شود. نتایج نشان داد که ازنظر ویژگی‌های ریزریخت‌شناسی تفاوت معنی‌داری بین دو فرم راش وجود ندارد.

INTRODUCTION

The geographical distribution of *Fagus* ranges discontinuously over most temperate areas of the Northern Hemisphere (Jones 1986) including eastern North America, Mexico, Europe, southwestern Asia, and East Asia, where it may dominate in mesic temperate broad-leaved forests (Cao 1995). Oriental beech or eastern beech (*Fagus orientalis* Lipsky) is indigenous to the Balkans in the west, through Anatolia (Asia Minor), to the Caucasus, northern Iran and Crimea (Atalay 1992; Kandemir & Kaya 2009).

Fagus orientalis is the most important species of the Hyrcanian forest of Iran, which accounts for approximately 17.6% of the total forest area, 30% of

the standing volume and 23.6% of the stem number. This shade tolerant species with high competition potential covers the north aspects of the middle altitudes of the Hyrcanian forests from west (Astara, Gilan province, border to Azerbaijan Republic) to east (Ziyarat Valley of Gorgan, Golestan province). It is the dominant tree between 700 and 1500 m a.s.l. where the air moisture is high and is covered with fog most of the year, especially within the growing season. It makes up pure and mixed stands with other noble hardwoods including velvet maple (*Acer velutinum* Boiss.), Caucasian alder (*Alnus subcordata* C. A. Mey.), large-leaved lime (*Tilia platyphyllus* Scop.) and common hornbeam (*Carpinus betulus* L.). However, individuals or small groups of beech could be found in altitudes around 300 and 2000 m a.s.l. Some old beech individuals can achieve high dimensions, up to 1 m diameter at breast height and 50 m high (Sagheb Talebi & al. 2014).

Fagus orientalis of Hyrcanian forests has been studied at the morphological (Parsa 1949; Menitsky 1971; Sabeti 1976) and genetic (Salehi Shanjani & al. 2003, 2010, 2011; Salehi Shanjani & Gömöry 2004; Gömöry & al. 2007) levels. For long time, native people and forest residents throughout Hyrcanian forests (Golestan, Mazandaran and Gilan provinces) have traditionally, considering the appearance of trees, recognized *F. orientalis* into two forms: black beech, and white beech. People of rural communities have been clearly able to distinguish between these two for a long time. Black beech is characterized with darker and broken bark; white beech with smoother light grey bark and light color wood. Till now, only one research has been done with subject of differences between black and white beeches, which considered the physical and mechanical properties of their woods (Rafeey 1997). Naderi (1988) has also mentioned the existence of differences in physical and mechanical properties of the wood between the two forms of beeches.

Micro-morphological characters are more constant and provide efficient, reliable and detailed data for distinguishing taxa. Micro-morphology of leaf and pollen grain of *Fagus* species have been studied (e.g. Hanks & Fairbrothers 1976; Praglowski 1982; Uzunova & Palamarev 1982; Jones 1986; Denk 2003; Cho & al. 2014). In a research project, we reviewed the genera of Fagaceae in Zagros, Arasbaran and Hyrcanian forests of Iran with emphasis on micro-morphological characteristics. In our studies, we tried to solve problems of systematic position of Fagaceae family in Iran. All data have been set out in detail in a series of publications (Panahi & al. 2011, 2012a, b, c, d). In this research, we are reporting the result of our observation on the genus *Fagus*. We did a complete survey on habitats of *F. orientalis* and micro-morphological patterns. We will try to evaluate some micro-morphological characteristics of leaf and pollen grains of black and white beeches to have more knowledge about variation in beech trees in Hyrcanian forests.

MATERIALS AND METHODS

The majority of white and black specimens used in this study were obtained during several field trips to areas according to their geographic ranges in the Hyrcanian forests in three province of Golestan, Mazandaran and Gilan (Fig. 1). In each province, at least 10 trees from two collections of black and white beech were chosen, separately in order to ensure about the constancy of pollen characters among different populations of certain entities. The collected samples were dried under room temperature and preserved as herbarium sheets. The voucher specimens are deposited in Herbarium of Research Institute of Forests and Rangelands of Iran (TARI). Furthermore other previously collected specimens of TARI were studied. Materials and collected data of some individual plant samples of all studied trees are listed in table 1.

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). Ten leaves from black beeches and the same number from white beeches were sampled for SEM (scanning electron microscope). Leaf samples measuring about 10 mm^2 were removed from the interval zone of each leaf and were mounted directly on aluminum stubs using double-sided adhesive tape. The number of each trichome type and stomatal density was counted on a standard area of 1 mm^2. Terminology used for trichome types is based on Jones (1986) and identification of wax layer types has been done according to Barthlott & al. (1998).

Fig. 1. White and black beeches, Shast Kalateh forest, Golestan province, 1300 m a.s.l. (Photo by Sagheb Talebi, 2016)

Table 1. Collection data of studied species in the forests of three different provinces of north Iran.

Taxon	Form	Collection data (Province, region, city or village)
Fagus orientalis Lipsky	black	Gilan, Asalem- Khalkhal road, Shondul nursery, 37˚39´48" N, 48˚49´20" E, alt. 1012 m, 2008, Panahi and Hasaninejad 101582 (TARI)
	white	Gilan, Asalem- Khalkhal road, Shondul nursery, 37˚39´48" N, 48˚49´20" E, alt. 1012 m, 2008, Panahi and Hasaninejad 101583 (TARI)
	black	Gilan, Asalem- Khalkhal road, Shondul nursery, 37˚40´15" N, 48˚49´24" E, alt. 972 m, 2008, Panahi and Hasaninejad 101584 (TARI)
	white	Gilan, Asalem- Khalkhal road, Shondul nursery, 37˚39´17" N, 48˚49´06" E, alt. 1130 m, 2008, Panahi and Hasaninejad 101585 (TARI)
	white	Golestan, Gorgan, Kordkuy, Deraznow, 36˚41´38" N, 54˚06´31" E, alt. 1260 m, 2008, Panahi and Hasaninejad 101588 (TARI)
	black	Golestan, Gorgan, Kordkuy, Deraznow, 36˚40´68" N, 54˚06´32" E, alt. 1715 m, 2008, Panahi and Hasaninejad 101590 (TARI)
	white	Golestan, Gorgan, Kordkuy, Deraznow, 36˚41´26" N, 54˚05´80" E, alt.1546 m, 2008, Panahi and Hasaninejad 101589 (TARI)
	black	Golestan, Gorgan, Kordkuy, Deraznow, 36˚40´36" N, 54˚07´49" E, alt. 2097 m, 2008, Panahi and Hasaninejad 101591(TARI)
	white	Mazandaran, Nowshahr, Kheirud Kenar Forest, Gorazbon, 36˚34´70" N, 51˚34´83" E, alt. 718 m, 2008, Panahi and Pourhashemi 102839 (TARI)
	black	Mazandaran, Nowshahr, Kheirud Kenar Forest, Gorazbon, 36˚34´68" N, 51˚34´81" E, alt. 718 m, 2008, Panahi and Pourhashemi 101581(TARI)
	black	Mazandaran, Nowshahr, Kheirud Kenar Forest, Gorazbon, 36˚34´73" N, 51˚34´85" E, alt. 730 m, 2008, Panahi and Pourhashemi 102840 (TARI)
	white	Mazandaran, Nowshahr, Kheirud Kenar Forest, Gorazbon, 36˚34´73" N, 51˚34´85" E, alt. 730 m, 2008, Panahi and Pourhashemi 102841(TARI)

Pollen grains of beech trees were collected from living plants. Pollen grains were prepared by the standard acetolysis method described by (Harley 1992), after which they were mounted in glycerin jelly and sealed with paraffin wax prior to LM observation. The micromorphological features and quantitative characters of pollen grains were studied by Vega2 /Tescan scanning electron microscope at an accelerating voltage of 15 KV and photographed by Olympus camera using a ×100 eyepiece. Measurements were made on 30 grains systematically scanning the slide for white and black beeches. Grains which lacerated or wrinkled were not measured. Terminology used for pollen shapes and features are based on Erdtman (1986), Punt & al. (1994), Halbritter & al. (2006) and Hesse & al. (2009). SEM micrographs were used mainly for studying the overall shape, type of sculpturing, and to get more detailed information on the structural, sculptural and suprasculptural patterns of pollen grains.

The mean, range and standard deviation (SD) were calculated for pollen grain and leaf variables. Normality distribution of variables was assessed by test of Anderson-Darling ($P < 0.01$) and independent samples t test was used to compare the mean values for each variable. The ratio of polar axis to equatorial diameter (P/E) was provided as an index of pollen shape (Faegri & Iversen 1964). The wall thickness of pollen grains was measured, too.

RESULTS

Fagus orientalis is a single-stemmed high-branch tree. The bark is smooth or furrowed. The black beech appears with dark grey and broken bark as well as reddish colorful wood, while white beech appears with smooth light grey bark and light color wood. Leaves shape are ovate to elliptic and sometimes asymmetric, dark green, turning yellow to light brown in autumn. The number of secondaries is between 8 and 14 (Figs. 2a, b, f, g, k, l). Leaf margin is usually wavy, untoothed or slightly toothed. Male flower are small and yellow with 12 stamens in two or three whorls (Figs. 2c, d, h, i, m, n). Female inflorescences are small and green and arranged solitarily in leaf axils (Figs. 2e, j, o). Fruit has bristle husk with edible nut.

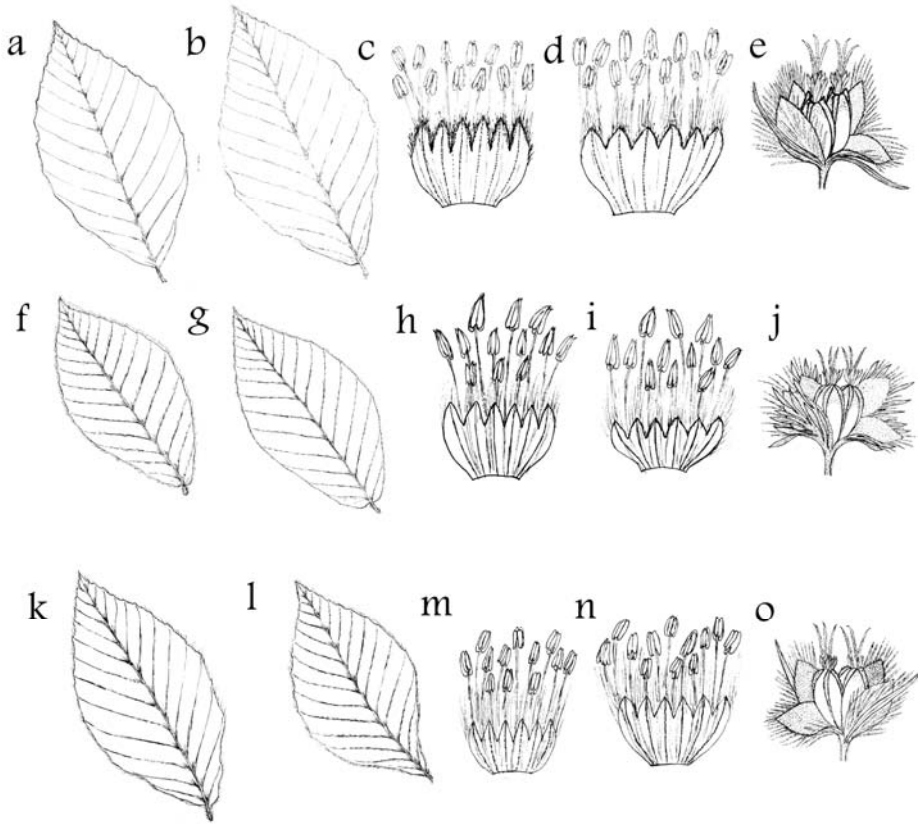

Fig. 2. Leaf, male and female inflorescences of the *F. orientalis*, Gilan province: white beech (a, c), black beech (b, d, e); Mazandaran province: white beech (f, h), black beech (g, i, j); Golestan province: white beech (k, m), black beech (l, n, o).

Pollen grain

The size of pollen grains observed in this study is categorized in medium (25-50 µm) and large (50-100 µm). In *Fagus* medium pollen grains are found, but most of pollen grains are large (45-63 µm). Pollen grains of examined specimens are single, isopolar, radially symmetrical, tricolporate. Colpi are medium length (Fig. 3a) with more or less acute apex. Three colpi run parallel to the polar axis and converging close to the polar ends (Fig. 3k). The geniculus is sometimes present (Fig. 3h). The wall thickness (Figs. 4c, d) is equal in mesocolpium and apocolpium ranging from 2-4 µm. Sexine is thicker than nexine. Light microscopic analyses have revealed that the shape of pollen grains ranges from oblate- spheroidal (P/E = 0.96, Fig. 3p) to prolate-spheroidal (P/E = 1.02; Figs. 3k, n).

The exine ornamentations in mesocolpium (Figs. 3h, j) and apocolpium (Fig. 3c) are mostly similar. Pollen surface is rough except near the colpi. Regarding to structural patterns of exine, vermiculate (rugulate) with small perforations in the tectum can be distinguished. These ornamentations which occur on the tectum may be fused sexine elements (Figs. 3l, m) or show loosely arranged sexine elements (Figs. 3o, q). Furthermore, structural patterns include perforations of varying density (dense, Figs. 3o, t; sparse, Figs. 3b, j) and varying distribution (regular, Fig. 3t; irregular, Figs. 3e, g; lineate, Figs. 3m, n, s).

Descriptive statistics of polar axis, equatorial diameter, P/E and wall thickness of pollen grains are summarized in table 2. The mean values of polar axis and equatorial diameter differ significantly among studied taxa ($p < 0.01$). On the average, the white beech has the highest values of polar axis and equatorial diameter meanwhile, wall thickness in the black type is more than that of in whites.

Trichome

Cuticle micromorphology of all specimens of *F. orientalis* was examined in the present study. Trichomes were present on the abaxial surface or on both surfaces of leaves, but trichome density and variability were more abundant on the abaxial leaf surface. The studied entities have three different trichome types as follows: Simple-uniseriate, solitary and conical. Simple-uniseriate type is thin-walled, unicellular (Figs. 5b, 6h, k) or multicellular and uniseriate (Figs. 5h, j, 6c, g) with different length (52-228 µm). This trichome type is observed on the abaxial surface in all studied specimens and is more abundant than two other types. Solitary type is single, long, usually straight and unicellular often thin-walled (Figs. 5c, e, i, n, p). These trichomes only occur on midrib and primary veins of all studied specimens. Unicellular conical trichome, that is thick-walled and erect (Figs. 5g, o), is observed on the veins and its distribution is less than other trichomes. There is essentially no difference between solitary and conical trichomes except trichome length. Papillae are absent on abaxial leaf surface (Figs. 5a, d, 6a, i).

Table 2. Descriptive statistics of polar axis, equatorial diameter, P/E and wall thickness of pollen grains in two different forms of studied beeches

Variable	Form	
	White	Black
	Mean ± SD	Mean ± SD
Polar axis (µm)	(54.4 ± 2.9)	(51.7 ± 3.6)
Equatorial diameter (µm)	(56.2 ± 2.3)	(51.9 ± 2.8)
P/E	0.97	0.99
Wall thickness (µm)	(2.5 ± 0.5)	(3± 0.6)

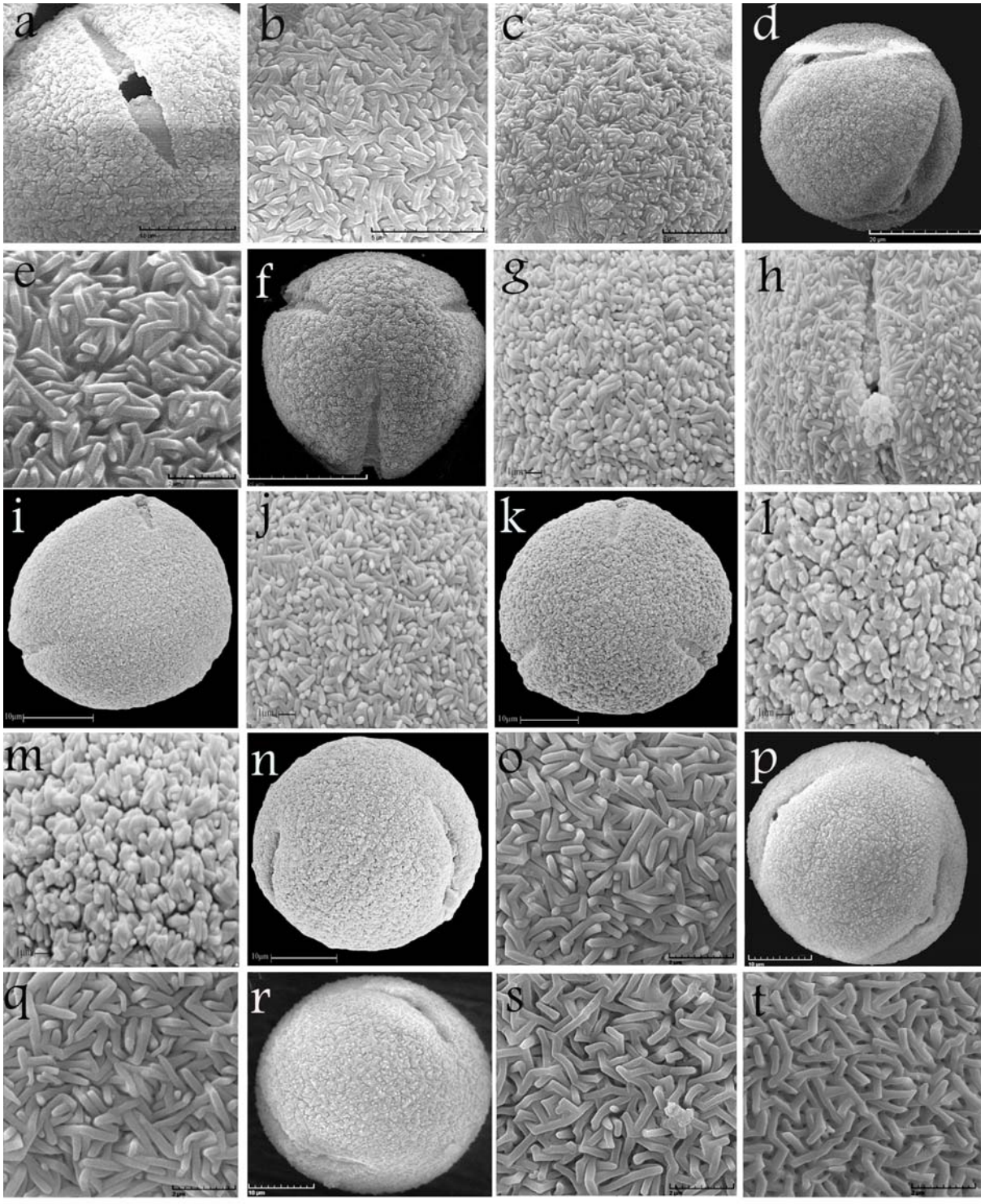

Fig. 3. SEM micrographs of pollen grains of the *F. orientalis*, Gilan province: white beech (a-c), black beech (d-f); Mazandaran province: white beech (g-j), black beech (k-n); Golestan province: white beech (o-q), black beech (r-t). Polar view: f, i, k; equatorial view: d, n, p, r; surface details in mesocolpium and apocolpium (a, b, c, e, g, h, j, l, m, o, q, s, t). Scale bar: 1 µm (g, h, j, l, m); 2 µm (e, o, q, s, t); 5 µm (b, c); 10 µm (a, i, k, n, p, r); 20 µm (d, f).

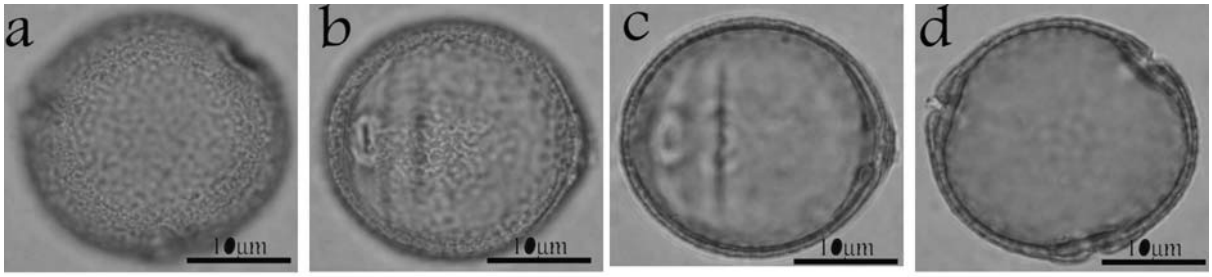

Fig. 4. LM micrographs of pollen grains of the *F. orientalis*, Polar view: a, d; equatorial view: b, c; Scale bar: 10µm.

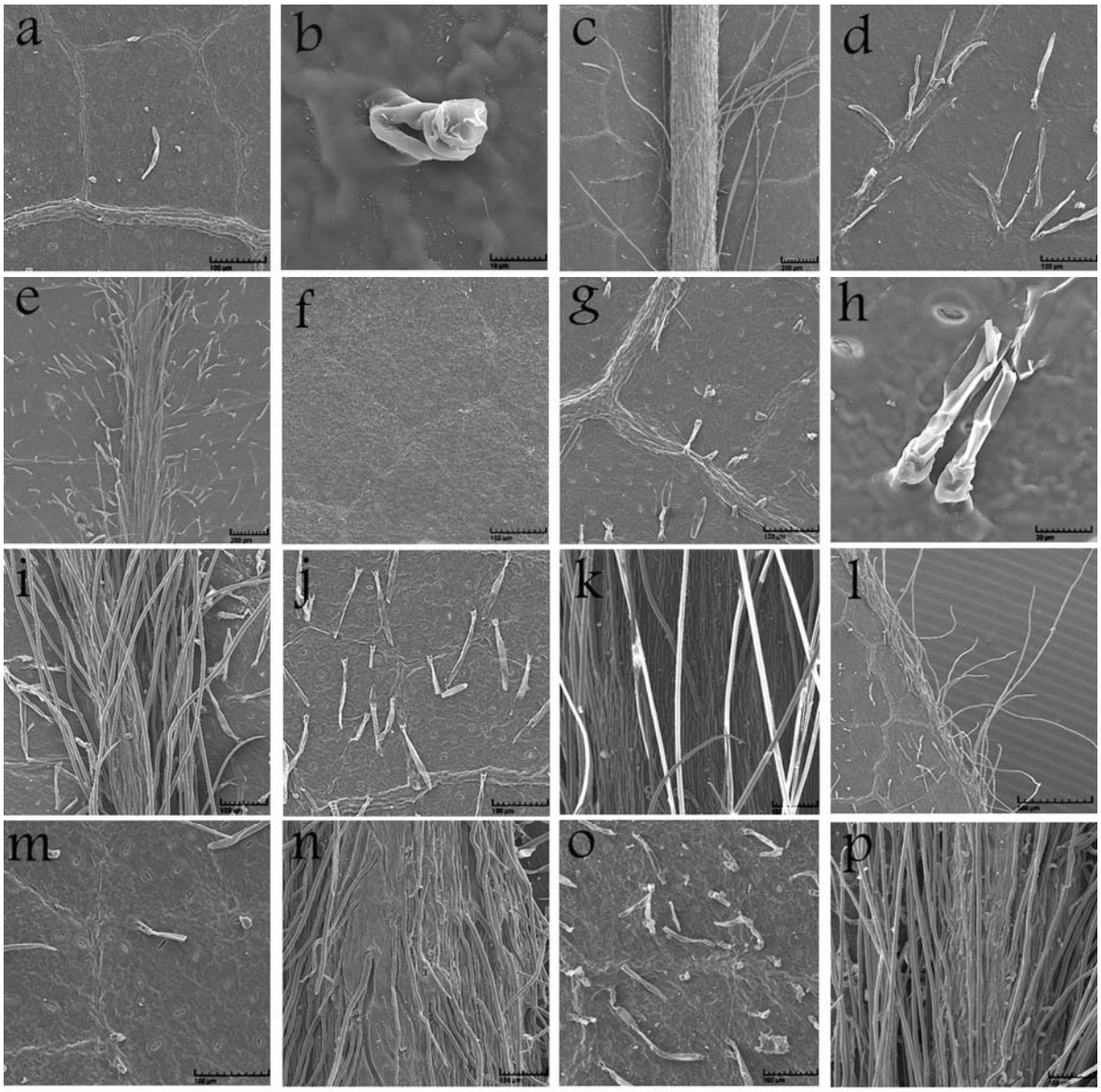

Fig. 5. SEM micrographs of trichomes of abaxial (a-e, g-p) and adaxial surface (f) in the studied entities; Gilan province: white beech (a-c, f), black beech (d-e); Mazandaran province: white beech (g-i), black beech (j-l); Golestan province: white beech (m-n), black beech (o-p). Scale bar: 10 µm (b); 20 µm (h); 100 µm (a, d, f-g, i-k, m-p); 200 µm (c, e); 500 µm (l).

Wax

Epicuticular wax of the abaxial surface is weakly developed in *F. orientalis* (Figs. 6e, l). The main type of epicuticular wax is recognized as smooth layers. Wax ornamentation is completely absent in young leaves.

Stomata

According to the arrangement of stomata subsidiary cells, *F. orientalis* has almost cyclocytic and randomly anomocytic type arrangement (Figs. 6a, i). All specimens have stomatal apparatus only on the abaxial surface (Fig 5f). We identified two stomata shapes: elliptical (Fig. 5j) and rounded (Fig. 6h). The rim of stomata is clearly elliptical in shape. The rims of the stomata are not covered by epicuticular waxes, so that the pore is visible (Figs. 6b, j). Size of stomata is almost large in *F. orientalis*. The average of long axis length is about 18-27 µm (Figs. 6f, j, k). The stomata are seen easily because of low density of trichomes (Figs. 5a, m). Stomata were raised above the epidermal surface in all cases.

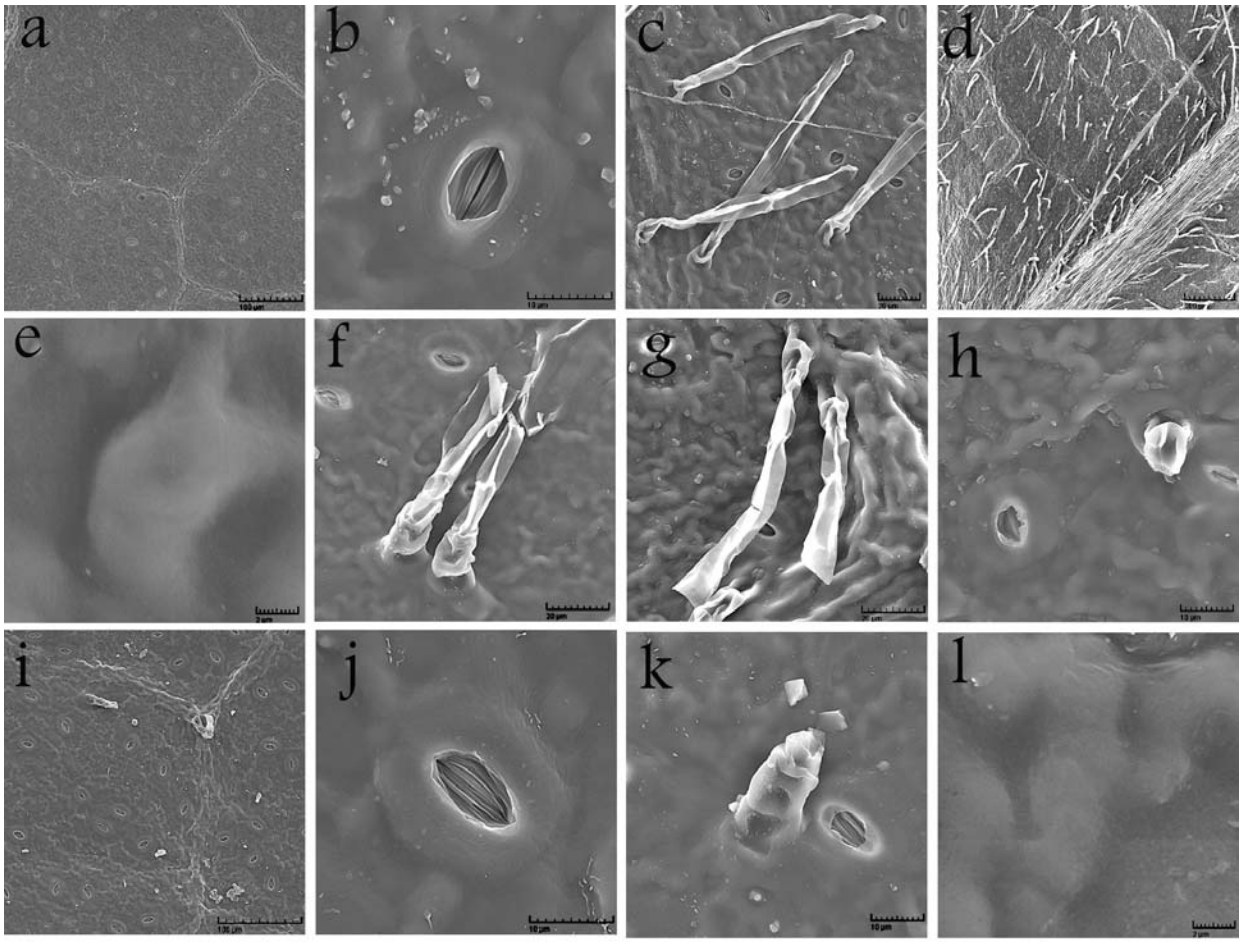

Fig. 6. SEM micrographs of trichomes, stomata and wax of abaxial surface in the studied entities; Gilan province: (a-b); Mazandaran province: (c-h); Golestan province: (i-l). Scale bar: 2 µm (e, l); 10 µm (b, h, j-k); 20 µm (c, f-g); 100 µm (a, i); 200 µm (d).

Descriptive statistics of leaf micromorphological characters are presented in table. 3. The mean values of all studied variables do not differ significantly among studied taxa ($p < 0.01$) except number of simple- uniseriate on abaxial surface.

Table 3. Descriptive statistics of leaf micro-morphological characters in two different beech forms among the provinces

Variable	Form	
	White	Black
	Mean ± SD	Mean ± SD
Number of simple-uniseriate on abaxial surface	7 ± 1	13± 1.4
Number of conical on abaxial surface	2.1 ± 0.7	2.1 ± 0.7
Length of simple-uniseriate (multicellular) rays on abaxial surface	104.1 ± 20.5	115.1 ± 25.8
Length of stomata (μm)	23 ± 1.8	21.8 ± 1
Width of stomata (μm)	17.1 ± 1	17.6 ± 1
Stomatal density (no.mm^2)	29.6 ± 1.6	29.7 ± 3.1

DISCUSSION

The form of broadleaved trees describe as decurrent in contrast to conifers as excurrent. Deciduous trees have single stem but the crown expands and the lateral branches grow as fast as the terminal leader (Barnes & al. 1998). The architectural model of beech tree based on Hallé & al. (1978) is known as Troll's model with sympodial form of shoots. In this research, micro-morphology of *F. orientalis* in Hyrcanian forests of Iran was studied. The objectives of this study was to conduct a detailed comparative characterization of foliar and pollen grain micro-morphological characters to evaluate these characters in white and black beeches. Despite extensive studies of the Fagaceae family from the genetic, biogeographic, phylogeographic level, macrofossils and macroevolutionary point of views, the taxonomic subdivision in this family is still controversial. The taxonomic history of *Fagus* in Europe and south-western Asia is complex, and opinions on the number of species in this area remain controversial (Denk & al. 2002). Denk (1999a, b) demonstrated that several geographical races of *Fagus* in Europe and south-western Asia are connected by morphological transitional forms, which do not occur as an ecocline from west to east. In Europe this name mostly refers to the *F. sylvatica* L. However, by moving toward east of Europe and south-west of Russia, this species is mixed with *F. orientalis*.

Some researches took place on the differences between the populations of beech in north of Iran, but only one study has been done on black and white beeches. Rafeey (1997) evaluated the difference of wood physical and mechanical properties of the two forms of beeches in Hyrcanian forests. He found the significant differences between these properties in black and white beeches. The black beech wood is reddish, and easy to work with hand tools but brittle. The white beech wood has higher density, lighter color, higher flexibility and good planed surface. He mentioned that several authors have discussed and these differences between beeches have been attributed to the ecological sources, but it is possible to find both of these two forms in similar ecological conditions (see Fig. 1). Naderi (1988) selected six individuals of beech in Veisar forest, Mazandaran province, to measure some mechanical properties of wood. He concluded that some individuals have obviously different mechanical properties. After more investigations and forest surveys, the results indicated that this discrepancy is due to white and black trees. Two forms of beech had significant differences in all measured properties through considered conditions.

Furthermore, genetic diversity of *F. orientalis* and its relationship with some physiological, biochemical and morphological characteristics in Hyrcanian forests of Iran were studied (Salehi Shanjani & al. 2010, Salehi Shanjani & Gömöry 2004). A considerable genetic multiplicity and diversity were found. Also population differentiation was moderate and differed significantly among forked and monopodial genotypes is obvious, but the results show that population genetic structures are also influenced by unpredictable factors such as wind direction at pollination and can be therefore, varied from year to year (Salehi Shanjani & al. 2003, 2011). These studies, did not mention the black and white beeches in the Hyrcanian forests.

Our research confirmed that there are minor differences in micro-morphological characters between white and black beeches in the Hyrcanian forests of Iran. Of course, more studies need to better identification of these beeches.

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