MORPHOLOGY AND MICROMORPHOLOGY OF THE GENUS FRAXINUS L. IN IRAN

M. Kaveh, A. Tavassoli, R. Azadi & F. Memariani

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Fraxinus L. is one of the 24 genera in Oleaceae family and comprises 43 species that are distributed in northern hemisphere. Two species: *F. excelsior* L. and *F. angustifolia* Vahl. are reported from Iran. These species are very similar on the basis of morphological characteristics which make their identification difficult. In order to overcome the ambiguity of these two species and their subspecific ranks, morphological and micromorphological traits of the species in 55 populations were studied. Forty nine qualitative and quantitative morphological characteristics were studied. Phenetic analysis was carried out using SPSS ver. 16 software and phenogram of the species considering morphological characters were plotted. Furthermore, PCA analysis was carried out and the most variable characters were determined. Factor analysis revealed the color of adaxial and abaxial surfaces of leaflets, color of winter buds, type of inflorescence, shape of fruit and its tip, and margin of leaflets are the most effective separating characters. According to descriptive analysis, three factors including the type of inflorescence, color of winter bud and shape of the margin serration of the leaflets are the most important ones. Micromorphological studies using scanning electron microscope, revealed two types of trichomes: acicular and capitate. Pollen grains in all specimens are monad and tricolpate. An identification key was prepared using morphological and micromorphological features.

Mona Kaveh, Akhtar Tavassoli (correspondence < tavstars@yahoo.com >) Department of Biology, Faculty of Science, Alzahra University, Tehran, Iran.- Rahman Azadi, Research Institute of Forests & Rangelands, P.O.Box 13185-116, Tehran, Iran.- Farshid Memariani, Department of Botany, Research Institute for Plant Sciences, Ferdowsi University of Mashhad, Mashhad, Iran.

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مورفولوژی و میکرو مورفولوژی جنس .Fraxinus L در ایران

منا کاوه،دانشجوی کارشناسی ارشد دانشکده زیست شناسی دانشگاه الزهرا (س) تهران

اختر توسلی، استادیار دانشکده زیست شناسی دانشگاه الزهرا (س) تهران

رحمان آزادی، عضو هیئت علمی موسسه تحقیقات جنگل ها و مراتع کشور

فرشید معماریانی، مربی گروه آموزشی و پژوهشی هرباریوم پژوهشکده گیاهی دانشگاه فردوسی مشهد

.Fraxinus L یا جنس ون یکی از ۲۴ جنس از خانواده Oleaceae است که با ۴۳ گونه که در نیمکره شمالی انتشار دارند. دو گونه F. excelsior L. و F. angustifolia Vahl. از این جنس در ایران انتشار دارند. از آنجایی که این دو گونه بسیار به یکدیگر شباهت دارند، برای رفع ابهام در تشخیص این دو گونه، صفات ریخت شناسی، ریز ریخت شناسی ۵۵ جمعیت آن مورد بررسی قرارگرفت. برای بررسی ویژگی های مورد نظر، از آنالیز فنتیک استفاده شد و فنوگرام این گونه ها برای ویژگی های ریخت شناسی نیز ترسیم گردید. و برای تعیین صفات با بیشترین تنوع از تحلیل مولفه های اصلی استفاده شد. در تحلیل فاکتورهای اصلی، نتایج نشان داد که رنگ سطح فوقانی و تحتانی برگچه ها، رنگ جوانه زمستانه، نوع گل آذین، شکل میوه و راس میوه و حاشیه برگچه ها موثرترین صفات هستند. بر اساس آنالیز های توصیفی، سه فاکتور: نوع گل آذین، رنگ جوانه زمستانه و شکل حاشیه برگچه ها بیشترین اهمیت را داشتند. بررسی های ریزریخت شناسی بر روی کرک ها در دو گونه برای اولین بار در ایران انجام شد. بر اساس بررسی ها ی انجام شده با میکروسکپ الکترونی نگاره (SEM) دو نوع کرک شناسایی گردید: کرک های خطی و سرسان. دانه های گرده در تمام نمونه های مورد بررسی موناد و سه شیاره بودند. کلید شناسایی بر اساس صفات ریخت شناسی و ریز ریخت شناسی ارائه گردید.

INTRODUCTION

Fraxinus L. is one of the 24 genera in the Olive family (Oleaceae) (Taylor 1945, Hinsinger 2010). It comprises 43 species distributed in temperate and subtropical areas of the northern hemisphere. The species are mainly trees, rarely shrubs, and often deciduous. There are also some evergreen shrubs adapted to arid environments (Wharf 2011). Plants from this genus are widely used for timber (Fraxigen 2005, Wallander 2008). The most important timbers produced by this family are from the Ashes (*Fraxinus*), of which the best known is *F. excelsior* L. Ash has a worldwide reputation for its toughness and is used for a great number of purposes (Metcalfe & Chalk 1983). Some species attract considerable attention for their medicinal properties and find application in the folk medicine, as well as in the contemporary medicine. Some species grow as garden plants and some are cultivated as ornamentals (Wallander 2008). The intraspecific variations in the leaf morphology have been the case of many synonyms in *Fraxinus* (Wallander 2008). Two species, *F. excelsior* L. and *F. angustifolia* Vahl are reported from Iran (Murray 1969; Azadi 2005). In this study, micromorphology of the petioles and the pollen characteristics of the species were studied to evaluate their potential value in definition of species and interspecific variation.

MATERIALS AND METHODS

Fresh materials and herbarium specimens were used in this study (table 1). Fifty five populations of the two species *F. excelsior* and *F. angustifolia* were investigated, using different floras (Boissier 1879; Parsa 1949; Vasil'ev 1952; Murray 1969; Grohmann 1974; Yaltirik 1978; Zohary 1978; Critopoulus 1980 and Azadi 2005). Fourty nine qualitative and quantitative characters (table 2 and 3) were used for morphological studies. In order to reveal the species relationships, we have used cluster analysis and principal component analysis (PCA) (Ingrouille 1986). To determine the most variable morphological characters among the studied species, factor analysis based on principal components analysis was performed. SPSS ver. 16 software was used for statistical analysis.

Numerical taxonomy

Qualitative characteristics initially encoded according to the multi-state method (table 2), and for quantitative traits (table 3), maximum, minimum and average of the numerical values were considered. Phenetic analysis was carried out via SPSS ver. 16 software and clustering analysis using Ward method. In order to determine the most variable morphological characters, factor analysis basis on principal components analysis (PCA) was performed (Ingrouille 1986).

Micromorphology

The petiole of species were studied (the species studied are marked with star symbol in the table 1. Petioles were mounted on carbon-coated double-sided tape, sputter-coated with gold and studied via a Phillips XL30 (30 kV) scanning electron microscope according to Hacking *et al*. (2007).

Palynology

The pollen samples were obtained mostly from freshly collected specimens. Fully mature anthers were removed from the specimens and prepared without acetolysis method (Erdtman 1952 & 1969). Morphological studies were performed on minimum 20 pollen grains for each taxon by an Olympus Light Microscope Model Bx51. Image tool software ver. 3 were used in measuring the quantitative characters. Qualitative (dispersal unit, type of pollen) and quantitative characteristics (pollen size, polar axis width, equatorial axis length, P/E, distance between colpi, exine thickness) were studied (The studied species are marked with two stars symbol in table 1).

RESULTS AND DISCUSSION

Morphology

Forty nine morphological characteristics of the 55 populations from two species: *F. excelsior* and *F. angustifolia* were studied and the average results for the two species are given in table 4.

The quantitative results according to Flora Iranica (Murray 1969), Flora of Turkey (Davis 1978) and Flora of Iran in Persian (Azadi 2005), showed that there are some ambiguities and conflictions in qualitative characteristics.

According to our results, acicular trichomes were visible on some parts of the herbarium materials, the situation of their presence are of great importance in the taxonomy of two Fraxinus species. Few low density groups of acicular trichomes are generally visible on the abaxial surface of the leaflets and at the beginning or periphery of the main vein in the two species, while several groups of high density trichomes were only observed on rachis, petioles and sometimes on the woody shoots of one subspecies of *F. excelsior* (*F. excelsior* subsp. *coriariifolia* (Scheele) E. Murry and two varieties of *F. angustifolia* (*F. angustifolia* subsp. *syriaca* var. *pilosa* Azadi and *F. angustifolia* subsp. *persica* var. *pubescens* Azadi).

Table 1. Specimens used in this study. TARI: Herbarium of Research Institute of Forests and Rangelands, Tehran, Iran. FUMH: Herbarium of Ferdowsi University of Mashhad, Mashhad, Iran. UTCANH: Herbarium of College of Agriculture and Natural Resources, Tehran University, Karaj, Iran. HSBU: Herbarium of Shahid Beheshti University, Tehran, Iran. YNH: Herbarium of Natural Resources of Yasouj, Yasouj, Iran. AUH: Herbarium of Alzahra University, Tehran, Iran.
* Specimens that have been studied for micromorphological study. ** Specimens that have been studied for palynological study.

No	Species	Locality
1	*Fraxinus angustifolia* ssp. *persica* var. *pubescens**	Iran, Yasouj, Near Dilgoon, 46433 TARI
2	*Fraxinus angustifolia* ssp. *persica* var. *persica**	Iran, Yasouj, toward Dilgoon, 81329 TARI
3	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Fars, Shiraz toward Ardakan, 31057 TARI
4	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Fars, Shiraz, Delou Mountain, 83649 TARI
5	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Fars, Dena Mt., Bijan Pass, 31270 TARI
6	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Fars, 20 km from Estahbanat toward Neyriz, 47133 TARI
7	*Fraxinus angustifolia* ssp. *syriaca* var. *pilosa**	Iran, Lorestan, toward Emarat, after Ti village, 81322 TARI
8	*Fraxinus angustifolia* ssp. *syriaca* var. *pilosa*	Iran, Lorestan, Doroud toward Emarat, 81318 TARI
9	*Fraxinus angustifolia* ssp. *syriaca* var. *pilosa**	Iran, Azarbayjan, 30 km from Piranshahr to Sardasht, 83280 TARI
10	*Fraxinus angustifolia* ssp. *syriaca* var. *pilosa*	Iran, Azarbayjan, Oroumiyeh toward Oshnaviyeh, 83277 TARI
11	*Fraxinus angustifolia* ssp. *syriaca* var. *pilosa*	Iran, Chaharmahal & Bakhtiari, Lordegan, 54729 TARI
12	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Isfahan, 50 km toward Semirom, 46033 TARI
13	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Fars, Kamfirouz, Moghimabad, 33316 FUMH
14	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Khorassan, East of Neyshabour, Dizbad-e Olia, left side of highlands , 27791 FUMH
15	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Khorassan, 10 km from Shadmehr toward Kashmar, Ghouch palang mountain, 26692 FUMH
16	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Khorassan, SW Boujnord, Chenaran, margin of river, 40381 FUMH
17	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Khorassan, Tabarkouh village, 23255 FUMH
18	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Khorassan, west of Mashhad, Zabetian county, 13232 FUMH
19	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca**	Iran, Khorassan, Hasan baghlou, 1200 m, 42911 UTCANH
20	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Azarbayjan, Silvana, Bardehsou, 1067 HSBU
21	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Azarbayjan, 12 km from Tordileh toward Dehtash, Alghi valley, 862120 HSBU
22	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Qom, 87369 HSBU
23	*Fraxinus angustifolia* ssp. *persica* var. *persica**	Iran, Yasouj, Ghalam mountain, Kookhdan, 3363 YNH
24	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Yasouj, Gachsaran, Shadegan, 3352 YNH
25	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Yasouj, Kookhdan, Deli valley, 3359 YNH
26	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Yasouj, Mehrian Pass, 3380 YNH
27	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Yasouj, Koohsarak village, 3332 YNH
28	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Yasouj, Mahlian bridge, 3327 YNH
29	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Yasouj, Moradi Pass, 3336 YNH
30	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca**	Iran, Yasouj, Dinar mountain, 3371 YNH
31	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Yasouj, Kookhdan, Deli valley, 3370 YNH
32	*Fraxinus angustifolia* ssp. *persica* var. *pubescens*	Iran, Yasouj, Sisakht, 3385 YNH
33	*Fraxinus angustifolia* ssp. *persica* var. *pubescens***	Iran, Yasouj, margin of abshar river, 3394 YNH
34	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Khorasan, Mashhad, Beheshte Reza, 89110 AUH
35	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Isfahan, Malekshahr, 89111 AUH
36	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Tehran, Vanak village, 89112 AUH
37	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Tehran, National Botanical Garden, 89113 AUH

Table 1. Continued.

No	Species	Locality
38	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Tehran, National Botanical Garden, 89114 AUH
39	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca***	Iran, Tehran, National Botanical Garden, 89115 AUH
40	*Fraxinus angustifolia* ssp. *syriaca* var. *syriaca*	Iran, Tehran, National Botanical Garden, 89116 AUH
41	*Fraxinus angustifolia* ssp. *persica* var. *syriaca*	Iran, Tehran, National Botanical Garden, 89117 AUH
42	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Tehran, National Botanical Garden, 89118 AUH
43	*Fraxinus angustifolia* ssp. *persica* var. *persica*	Iran, Tehran, National Botanical Garden, 89120 AUH
44	*Fraxinus angustifolia* ssp. *persica* var. *pubescens**	Iran, Tehran, National Botanical Garden, 89119 AUH
45	*Fraxinus excelsior* ssp. *coriariifolia**,**	Iran, Khorassan, west of Bojnord, Darkash, Kani Mokhtar, 35604 FUMH
46	*Fraxinus excelsior* ssp. *coriariifolia*	Iran, Khorassan, west of Bojnord, between Havar & Showghan, Aghbaba, 33083 FUMH
47	*Fraxinus excelsior* ssp. *excelsior*	Iran, Khorassan, southwest of Bojnord, Rein valley towards Garmak, 39140 FUMH
48	*Fraxinus excelsior* ssp. *coriariifolia*	Iran, Khorassan, southwest of Bojnord, Rein valley towards Garmak, 38136 FUMH
49	*Fraxinus excelsior* ssp. *excelsior*	Iran, Khorassan, west of Bojnurd, Ghorkhod, Kanisalan highlands, 44444 FUMH
50	*Fraxinus excelsior* ssp. *excelsior**	Iran, Khorassan, west of Bojnord, Zaman Soofi village, 35171 FUMH
51	*Fraxinus excelsior* ssp. *excelsior*	Iran, Khorassan, Ghoochan, 4301 UTCANH
52	*Fraxinus excelsior* ssp. *excelsior**	Iran, Golestan, Gorgan, National park, 82277 HSBU
53	*Fraxinus excelsior* ssp. *excelsior***	Iran, Tehran, National Botanical Garden, 89121 AUH
54	*Fraxinus excelsior* ssp. *excelsior*	Iran, Tehran, National Botanical Garden, 89122 AUH
55	*Fraxinus excelsior* ssp. *coriariifolia*	Iran, Tehran, National Botanical Garden, 89123 AUH

Table 2. Investigated qualitative characters.

Characters	Coded character states			
	1	2	3	4
Color of adaxial surface of leaflet	green-gray	dark green	light green	gray
Color of abaxial surface of leaflet	green-gray	dark green	light green	gray
Shape of leaflet	ovate	ellipsoid	oblong	
Shape of leaflet apex	rounded	acute		
Shape of leaflet base	rounded	attenuate		
Shape of leaflet margin	fine serrate	coarse serrate	almost entire or irregular serrate	
Location of trichomes	leaflet, rachis, petiole	sometimes along the midrib		
Shape of fruit	oblong	ellipsoid	ovate	linear

Table 3. Investigated quantitative characters in morphological study.

Length of leaf	Distance of leaflet pairs
Length of petiole	Length of rachis
Length of leaflet	Width of leaflet
Number of leaflets	Dent angle of leaflet
Length of winter bud	Width of winter bud
Length of fruit	Width of fruit
Length of inflorescence	

Table 4. Morphological characters of *F. excelsior* and *F. angustifolia*.

No.	Characters	*F. excelsior*	*F. angustifolia*
1	Maximum length of leaf	40 cm	34 cm
2	Minimum length of leaf	10 cm	4.5 cm
3	Average length of leaf	16.75 cm	24.62 cm
4	Maximum length of rachis	20 cm	19 cm
5	Minimum length of rachis	9 cm	3 cm
6	Average length of rachis	15 cm	10.56 cm
7	Maximum distance between pairs of leaflets	4 cm	6 cm
8	Minimum distance between pairs of leaflets	1.3 cm	1.8 cm
9	Average distance between pairs of leaflets	2.5 cm	3.48 cm
10	Maximum length of petiole	7 cm	9 cm
11	Minimum length of petiole	1.5 cm	2 cm
12	Average length of petiole	4.6 cm	5.06 cm
13	Maximum length of leaflet	8 cm	14 cm
14	Minimum length of leaflet	2.3 cm	1 cm
15	Average length of leaflet	5.7 cm	5.56 cm
16	Maximum width of leaflet	3.3 cm	4.2 cm
17	Minimum width of leaflet	1 cm	0.5 cm
18	Average width of leaflet	2.15 cm	2.12 cm
19	Maximum number of leaflets	11	11
20	Minimum number of leaflets	7	3
21	Shape of serration	Fine serrate	Coarse serrate to nearly Toothless
22	Maximum angle of the serration	81°	129.61°
23	Minimum angle of the serration	21°	37°
24	Average angle of the serration	51.5°	77.37°
25	Color of adaxial surface of leaflet	Light green to dark green	Light to dark green sometimes green-gray
26	Color of abaxial surface of leaflet	Blur, light green-gray	Green-gray to dark green
27	General shape of leaflet	Ovate to oblong	Ellipsoide to oblong
28	Apex of leaflet	Rounded almost acute	Acute
29	bottom of leaflet	Rounded almost attenuate	Attenuate
30	Existence trichome on the petiole	In one subspecies	In two varieties
31	Maximum length of winter bud	5.5 mm	5.9 mm
32	Minimum length of winter bud	0.1 mm	0.5 mm
33	Average length of winter bud	2.99 mm	2.1 mm
34	Maximum width of winter bud	5.2 mm	4.3 mm
35	Minimum width of winter bud	0.1 mm	1.2 mm
36	Average width of winter bud	2.95 mm	1.7 mm
37	Color of winter bud	Black	Dark brown
38	Type of inflorescence	Panicle	Raceme
39	Shape of fruit	Oblong to ellipsoid	Oblong to ellipsoid and ovate
40	Apex of fruit	Retuse, obtuse	Obtuse, mucronate and acute
41	Maximum length of fruit	4.6 cm	4 cm
42	Minimum length of fruit	2.5 cm	1 cm
43	Average length of fruit	3.65 cm	2.8 cm
44	Maximum width of fruit	1 cm	2 cm
45	Minimum width of fruit	0.5 cm	0.2 cm
46	Average width of fruit	0.75 cm	1.1 cm
47	Maximum length of inflorescence	13 cm	18 cm
48	Minimum length of inflorescence	4 cm	3.5 cm
49	Average length of inflorescence	8.75 cm	7.9 cm

Table 5. Preliminary results of the factor analysis based on qualitative morphological characters.

Factor	percentage of variance	percentage of cumulative
1	60.25	60.25
2	21.34	81.59
3	11.23	92.83

Table 6. The values of the first three factors of the factor analysis based on qualitative morphological characters.

Characters	Factor 1	Factor 2	Factor 3
Color of adaxial surfaces of leaflets	0.8	-	-
Color of abaxial surfaces of leaflets	0.9	-	-
General shape of leaflet	-	0.7	-
leaflet base	-	0.8	-
Color of winter bud	0.9	-	-
Type of inflorescence	0.9	-	-
General shape of fruit	0.7	-	-
fruit apex	0.9	-	-
leaflets margin	0.9	-	-
Existence of trichome on the petiole	-	-	0.6

Leaflets margins show some differences in studied species; F. excelsior has fine serration on its leaflet margins, while there are two types of serration in F. angustifolia: coarse serrate margin in subsp. syriaca (Boiss.) Yalt. and entire, sometimes with irregular serration close to the tip of the leaflet in subsp. *persica* (Boiss.) Azadi (fig.1).

The color of winter bud in *F. excelsior* is black, while in *F. angustifolia* is dark brown (fig. 2).

Inflorescence is compound in *F. excelsior* but simple in *F. angustifolia* (fig. 3).

Shape of fruit is oblong to ellipsoid in *F. excelsior*, while ovate, ellipsoid to oblong in *F. angustifolia*. Fruit in *F. excelsior* has a retuse and obtuse apex while the apex of fruit in *F. angustifolia* is acute and mucronate, but sometimes obtuse apex was observed (fig. 4).

Typically, samara has one seed but in some rare cases two or three seeds were observed in *Fraxinus*. In the very rare cases, especially in *F. angustifolia*, triangular samara was observed instead of the flatted samara (fig. 5).

The sizes of all morphological features of *Fraxinus* are almost in agreement with earlier report by Azadi (2005). Among the regional Floras, the morphological features recorded in the Flora of Turkey (Yaltirik 1978) and Flora of USSR (Vasil'ev 1952) are more compatible with our results. Fraxigen (2005) reported that the type of inflorescence is the clearest diagnostic character in these two species and the color of winter buds and the shape of serration in leaflet margins are the next important characters.

Factor analysis revealed that the first three factors comprise 92.83% of the total variation (table 5 & 6). The PCA analysis, revealed the color of adaxial and abaxial surfaces of leaflets, color of winter bud, type of inflorescence, shape of fruit, shape of fruit tip and shape of serration in leaflets are the most effective characters (table 6).

Based on qualitative morphological characteristics, the phenogram (diagram 1) was divided into two original clusters in the linkage distance 25. The first cluster that included the four varieties of *F. angustifolia*; sub-divided into two sub-clusters. In the first sub-cluster, 5 (*F. angustifolia* subsp. *persica* var. *persica*) and 6 (*F. angustifolia* subsp. *persica* var. *pubescens*) pertaining to *F. angustifolia* subsp. *persica*, located close to each other. Furthermore two varieties 3 (*F. angustifolia* subsp. *syriaca* var. *syriaca*) and 4 (*F. angustifolia* subsp. *syriaca* var. *pilosa*) which belong to the *F. angustifolia* subsp. *syriaca*, are also located adjacent to each other. In turn, the 2nd original cluster is divided into two subspecies of *F. excelsior*: 1 (*F. excelsior* subsp. *excelsior*) and 2 (*F. excelsior* subsp. *coriariifolia*) which are located adjacent to each other. The ordination of species based on PCA is also highly compatible with the related phenogram (diagram 2).

According to Wallander (2008), there is a high variation in the morphology of the leaf in *Fraxinus* including shape, texture, number of leaflets, leaflet margin, petiole length, indumentums, epidermal papillae, rachis wings, etc. Wharf (2011) reported the great variability in the leaf morphology can be regarded as a sign of individual adaptation against environmental conditions. According to our results, qualitative morphological characters are more stable and reliable as diagnostic characters. Jarni *et al*. (2010) reported in *F. angustifolia*, that growing in shade typically have larger leaves than those growing in sun, and also they have broader and shorter-pointed leaflets, in these features the leaves of *F. angustifolia* are very similar to the leaves of *F. excelsior*, consequently, it is very difficult to distinguish between them and they are often misidentified. Jarni *et al*. (2010) also reported that the most of the distinguishing traits (fruits, flowers, shoots) are in the upper canopy layers whereas the ground traits are very variable and cannot be reliably used for identification. In the case of color of winter buds, the experience has proved sometimes eye error is possible and it is difficult to distinguish dark brown from black, then it causes ambiguity in distinguishing the two species. Among the characters, type of inflorescence is

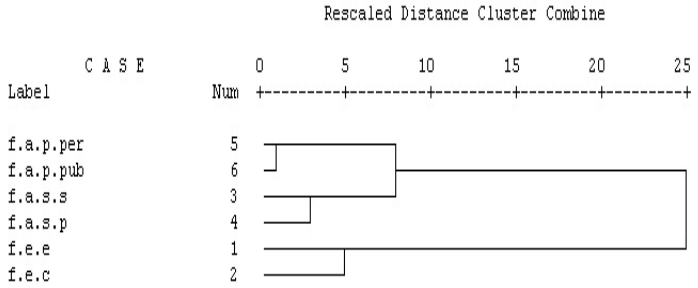

Diagram 1. Phenogram of clustering based on qualitative morphological characters.

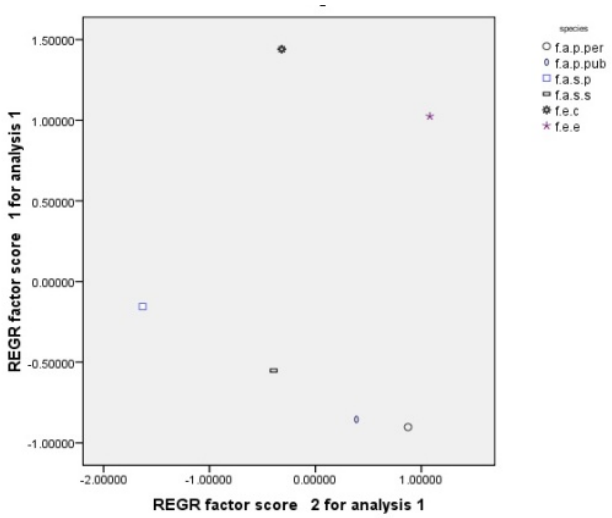

Diagram 2. Ordination based on qualitative morphological characters.

the absolute character and reliable to recognize. Also according to Fraxigen (2005) the clearest distinction is in the inflorescence type (figs. 6&7).

Palynology

Pollen grains of four subspecies of the two species are all monad and tricolpate. They are all similar in terms of qualitative but minor differences in quantitative traits and there are not significant differences among them (fig. 8, table 7).

Trichome micromorphology

Two types of capitate and acicular trichomes were observed on petioles of the specimens studied. capitate trichomes were observed on all specimens of the two species. Furthermore, *F. excelsior* subsp. *coriariifolia*, *F. angustifolia* subsp. *syriaca* var. *pilosa* and *F. angustifolia* subsp. *persica* var. *pubescens* had much higher density of trichomes on their petioles. Acicular trichomes of *F. angustifolia* subsp. *syriaca* var. *pilosa* seemed to be longer than those of other taxa (fig. 9) Kremer *et al.* (2008) reported capitate trichomes in all *Fraxinus* species, which are of the same shape on the main and lateral veins of both abaxial and adaxial leaf surfaces, and the acicular trichomes may be very rarely observed in the glabrous phase of both species.

According to results, distinct subspecies and varieties (including *F. angustifolia* subsp. *syriaca* var. *syriaca* and *F. angustifolia* subsp. *syriaca* var. *pilosa* and also *F. angustifolia* subsp. *persica* var. *persica* and *F. angustifolia* subsp. *persica* var. *pubescens*) were separated based on morphological and micromorphological characteristics. This confirms the earlier report by Azadi (2005). However, WCSP (2013) considered the varieties as synonyms of subspecific taxa i.e. subsp. *syriaca* and subsp. *persica*.

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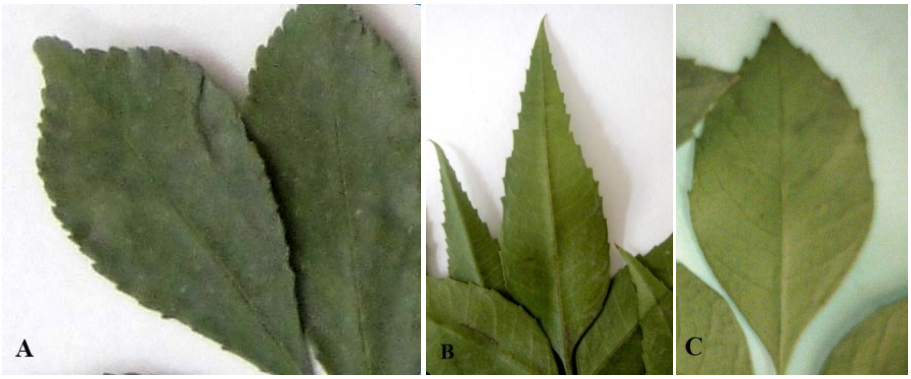

Fig. 1: Leaflet margin in A, *F. excelsior*; B, *F. angustifolia* subsp. *syriaca* and C, *F. angustifolia* subsp. *Persica*.

Fig. 2. A, Black winter bud in *F. excelsior* and B, dark brown winter bud in *F. angustifolia*.

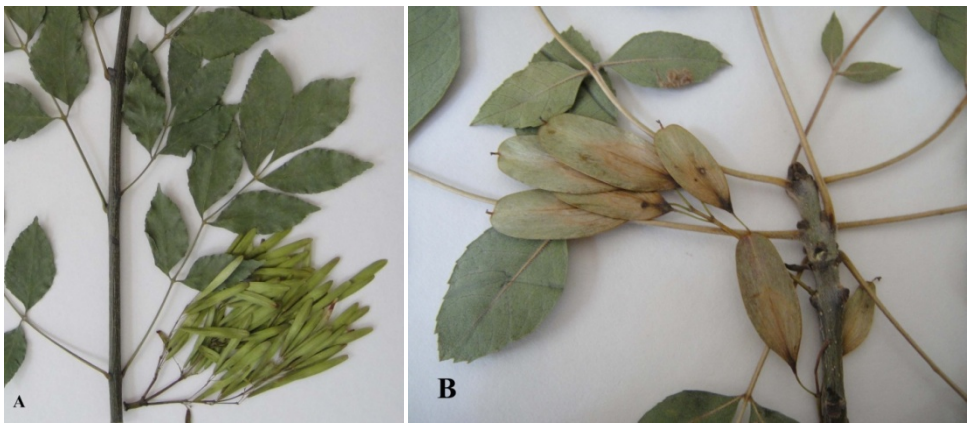

Fig. 3. A, panicle inflorescence in *F. excelsior* and B, raceme inflorescence in *F. angustifolia*.

Fig. 4. A, Fruit with retuse apex, B, obtuse apex in *F. excelsior*; C-D, acute or mucronate apex in *F. angustifolia*.

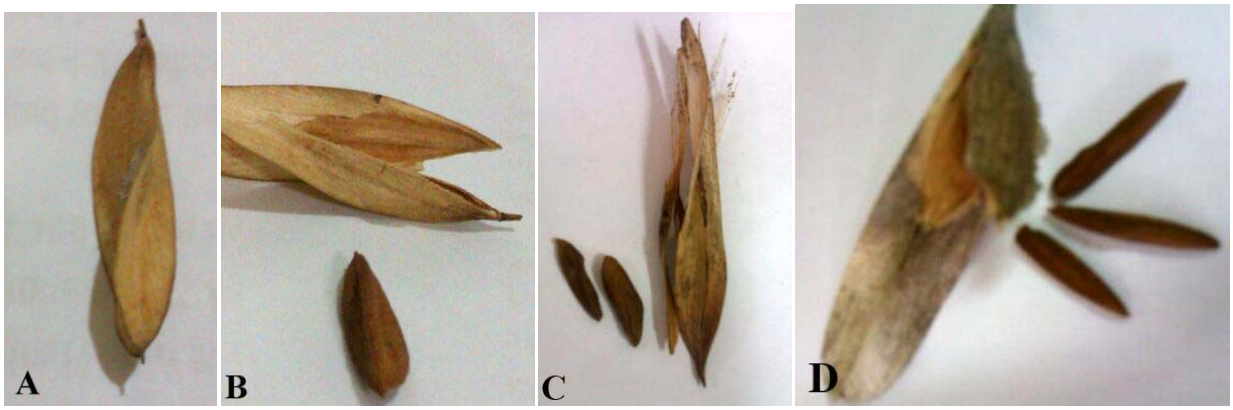

Fig. 5. Rare samaras with two or three seeds and with the triangular shape in *F.angustifolia* (89112AUH; 89116AUH; 89111AUH).

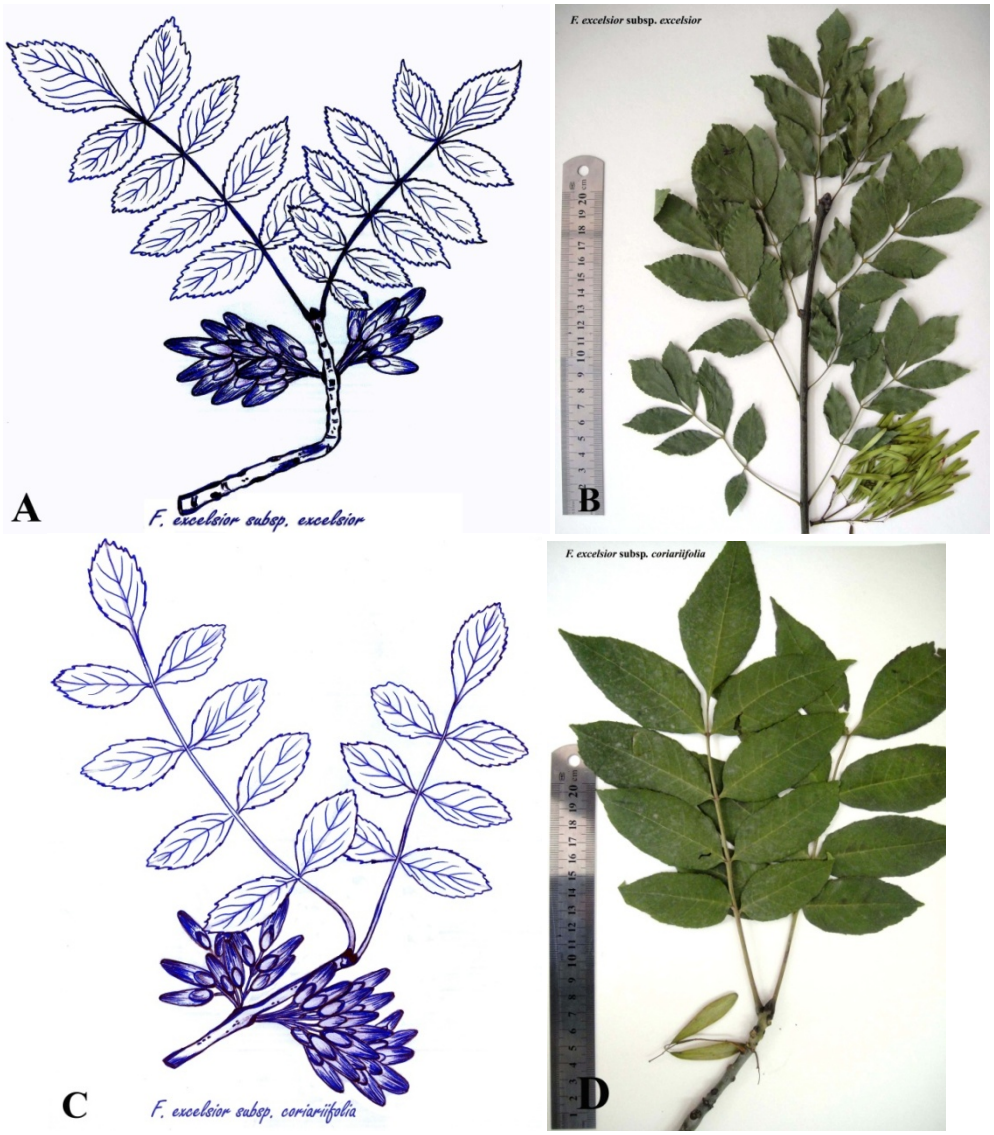

Fig. 6. A- B, *F. excelsior* subsp. *excelsior*; C- D, *F. excelsior* subsp. *coriariifolia*.

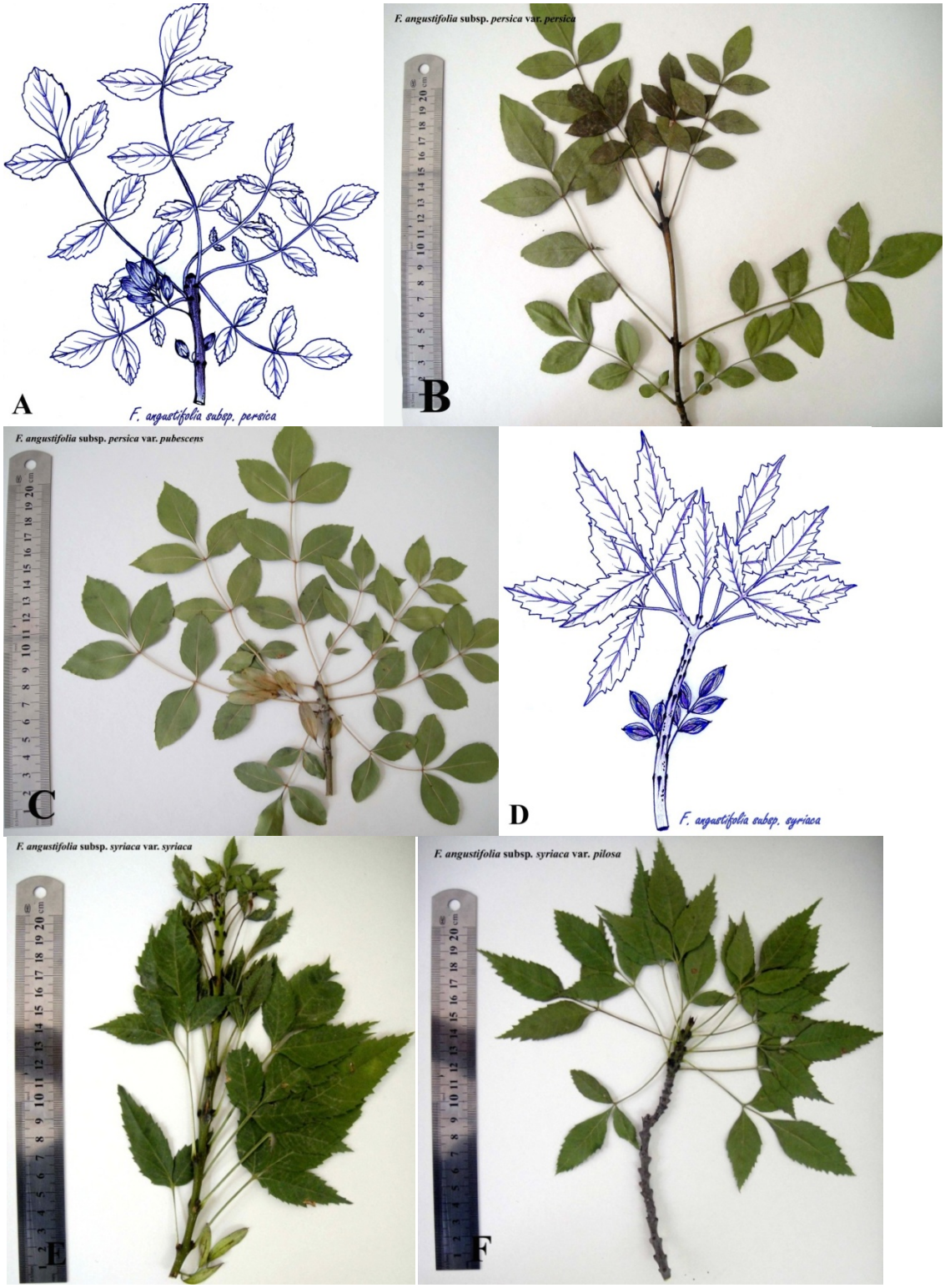

Fig. 7. A, *F. angustifolia* subsp. *persica*; B, *F. angustifolia* subsp. *persica* var. *persica*; C, *F. angustifolia* subsp. *persica* var. *pubescens*; D, *F. angustifolia* subsp. *syriaca*; E, *F. angustifolia* subsp. *syriaca* var. *syriaca*; F, *F. angustifolia* subsp. *syriaca* var. *pilosa*.

Table 7. Qualitative and quantitative micro-morphological characters of pollen of four subspecies of *Fraxinus*.

Subspesies / Character	*Fraxinus excelsior* ssp. *excelsior*	*Fraxinus excelsior* ssp. *coriariifolia*	*Fraxinus angustifolia* ssp. *syriaca*	*Fraxinus angustifolia* ssp. *persica*
Polar axis length	29.56 µ	28.14 µ	37.23 µ	26.54 µ
Equatorial axis length	38.80 µ	36.16 µ	43.79 µ	41.66 µ
P/E	0.76	0.77	0.85	0.63
Distance between colpi	21.11 µ	21.42 µ	31.10 µ	27.24 µ
Exine thickness	1.66 µ	1.46 µ	1.67 µ	1.49 µ

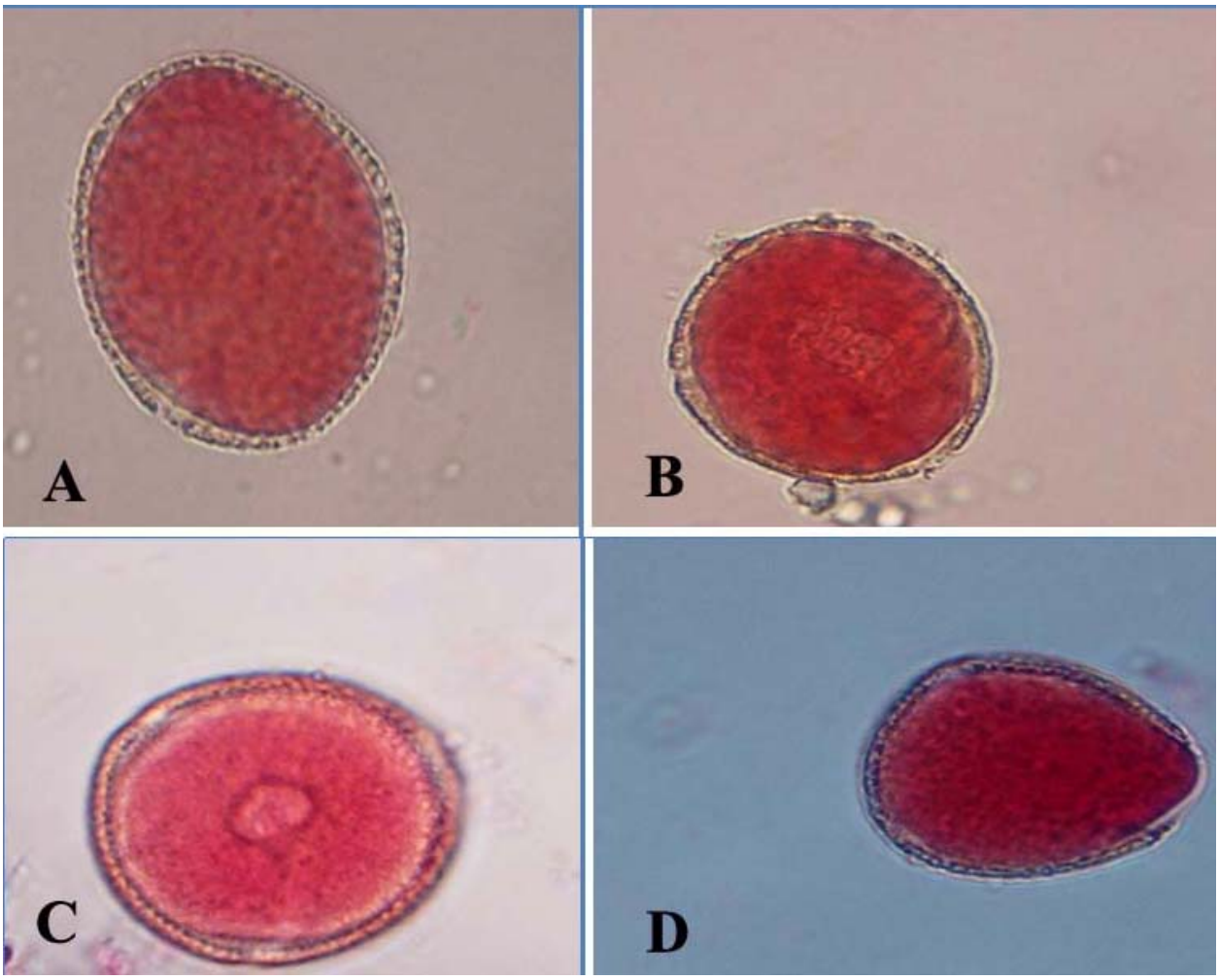

Fig. 8. Polar view of pollen grains in A, *F. excelsior* subsp. *excelsior*; B, *F. excelsior* subsp. *coriariifolia*; C, *F. angustifolia* subsp. *syriaca*; D, *F. angustifolia* subsp. *persica*.

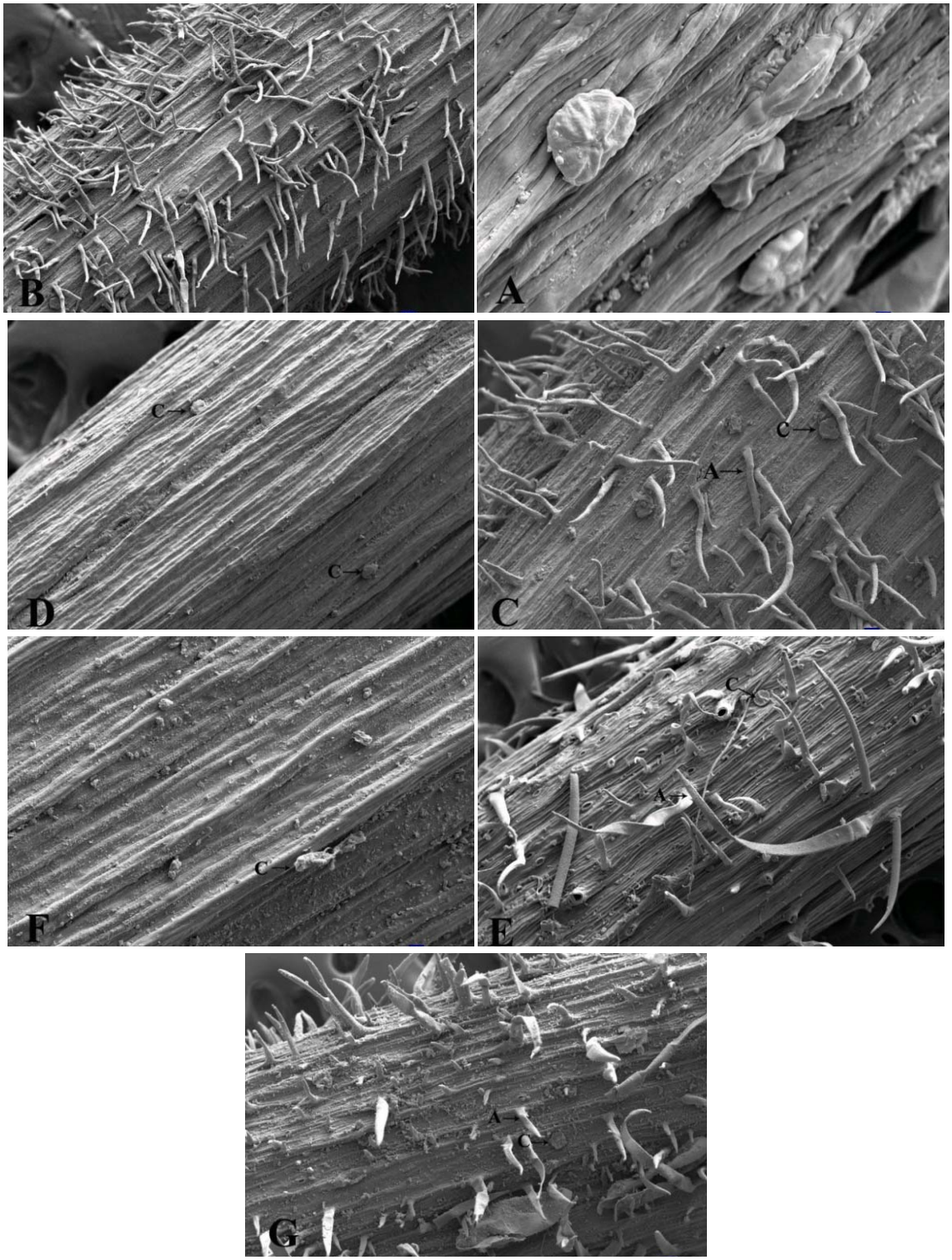

Fig. 9. A, Capitate trichomes in *F. excelsior* subsp. *excelsior*; B, acicular trichomes in *F. excelsior* subsp. *coriariifolia*; C, acicular and capitates trichomes in *F. excelsior* subsp. *coriariifolia*; D, Capitate trichomes in *F. angustifolia* subsp. *syriaca* var. *syriaca*; E, acicular and capitate trichomes in *F. angustifolia* subsp. *syriaca* var. pilosa; F, capitate trichomes in F. angustifolia subsp. persica var. persica; G, acicular and capitate trichomes in *F. angustifolia* subsp. *persica* var. *pubescens.*

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