

POLLEN MORPHOLOGY OF THE GENUS *CARPINUS* L. (CORYLACEAE) IN IRAN

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Pollen morphology of four taxa of the genus *Carpinus* growing in Iran was examined, using light (LM) and scanning electron microscopy (SEM). The main aim of this study is to determine the pollen morphology of this genus in Iran and finding the pollen characteristics. The pollen grains are triporate, tetraporate and pentaporate and more or less subprolate. The shape of pores are more or less circular, the surface sculpture of exine is irregularly cone-shaped and the apex is pointed. *C. orientalis* Miller subsp. *macrocarpa* (Willk.) Browicz and *C. betulus* var. *parva* have the largest and smallest pollen size (mean = $35.18 \pm 3.96 \mu\text{m}$) and (mean = $24.95 \pm 0.18 \mu\text{m}$), respectively.

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مورفولوژی گرده جنس ممرز (Corylaceae) در ایران

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مورفولوژی دانه گرده چهار تاکسون از جنس ممرز در ایران با استفاده از میکروسکوپ نوری و الکترونی مورد مطالعه قرار گرفت. هدف از مطالعه بررسی ریخت شناسی دانه گرده جنس ممرز و یافتن صفات مناسب در خصوص گرده شناسی این جنس در ایران است. دانه‌های گرده این جنس سه و چهار یا پنج منفذی و کم و بیش نیمه کشیده هستند. شکل منفذ کم و بیش گرد، تزئینات سطح اگزین مخروطی نامنظم و در انتها نوک‌دار است. *C. betulus* var. *parva* و *Carpinus orientalis* subsp. *macrocarpa* به ترتیب دارای بزرگترین و کوچکترین دانه گرده هستند (35.18 ± 3.96 و 24.95 ± 0.18 میکرومتر).

Introduction

Betulaceae is divided into two subfamilies (*Coryloideae* and *Betuloideae*). *Coryloideae* comprises ca. 45 species classified in 4 genera: *Carpinus* L., *Corylus* L., *Ostrya* Scopoli and *Ostryopsis* Decne. *Carpinus* is the largest genus of *Coryloideae* with about 35 species. The genus has an intercontinental disjunct distribution in Europe, North America and Eastern

Asia. *Coryloideae* have been supported as a monophyletic group (Bousquet et al., 1992; Chen & Zhang, 1991; Yoo & Wen, 2007). The subfamily is defined based on several characteristics including nutlets without lateral wings, vessels without spiral thickenings, absence of tracheids and pollen without arci (Thorne, 1992; Mabberley, 1997; Chen, et al., 1999). Chen (1991) studied pollen of 6 genera of

Table 1. Material used for pollen morphology of taxa of the genus *Carpinus* in Iran.

Taxon	Locality	Collector	Herbarium and Voucher no.
<i>C. betulus</i> L. var. <i>betulus</i>	Golestan: Aliabad, Zaringol, 1180 m, 10.2.2009	Akhondnejad	HNBG, 10677
<i>C. betulus</i> L. var. <i>parva</i> O. Radde	Eastern Azerbaijan: Arasbaran Biosphere Reserve, intersection village Tolikan, 1150 m, 7.3.2009	Akhondnejad	HNBG, 10678
<i>C. orientalis</i> Mill. subsp. <i>macrocarpa</i> (Willk.) Browicz	Gilan: Hashtpar, Karganrood, 333 m, 10.3.2009	Akhondnejad	HNBG, 10682
<i>C. orientalis</i> subsp. <i>orientalis</i>	Mazandaran: Amol, Chamestan, 1720 m, 12.3.2009	Akhondnejad	HNBG, 10681

Betulaceae in China and resulted in favour of the Abbe (1974), which divided *Betulaceae* in 3 tribes: tribe *Betuleae* (*Alnus* and *Betula*), tribe *Coryleae* (*Corylus*) and tribe *Carpineae* (*Ostryopsis*, *Carpinus* and *Ostrya*). *Betulaceae* and *Corylaceae* pollen grains have been widely investigated in palynological investigations concerned with such topics as allergy (El-Ghazaly et al., 1995; Henriksen et al., 1996), pollen exine substructure (Claugher & Rowley, 1987), pollen wall ontogeny (Dunbar & Rowley, 1984) as well as pollen production and aerobiology (Spieksma et al., 1995). The terminology follows those recommended by Punt et al. (2007) at level of light microscope, it is not always possible to identify individual dispersed pollen further than the generic level although subgroup can be recognized within some types. There aren't adequate evidences that scanning electron microscopy can show fine details of ornamentation in all species. The studies using transmission electron microscopy have provided a finer level of detail concerning aperture (Kedves & Pardutz, 1983), although it does not provide a practical method to identification of dispersed pollen grains.

The family *Betulaceae* and *Corylaceae* will be considered together due to the similarities between their pollen grains and their close systematic affinities. This account is also cross referenced to that of *Myricaceae* (Punt et al, 2007) because of the strong palynological similarities (Edward, 1981) and they usually appears close together in general pollen identification keys (Moor et al., 1991). However, pollen grains of *Carpinus* are more rounded and the exine is thinner. *Carpinus* can readily be distinguished from the other pollen types of *Betulaceae* and *Corylaceae* by its larger size (but there are problems with the size of pollen because size of pollen grains varies within a sample derived from a single catkin (Blackmore et al., 2003)).

Data on pollen morphology of *Carpinus* are lacking for the Iranian species. The aim of the present research was to study the diversity and range of variation of the pollen morphological characteristics of the genus

Carpinus in Iran and to check if these data improves the taxonomy of the genus. According to Browicz in Flora Iranica (1972), two species of *Carpinus* exist in Iran: *C. betulus* L. with two varieties: *C. betulus* var. *betulus* and *C. betulus* var. *parva* O. Radde and *C. orientalis* Miller with two subspecies: *C. orientalis* subsp. *macrocarpa* (Willk.) Browicz and *C. orientalis* subsp. *orientalis*. According to Sabeti (1972), four species of *Carpinus* exist in Iran: *C. betulus*, *C. macrocarpa* Willk., *C. orientalis* and *C. schuschaensis* H. Winkl. Ghahraman (2004) presented two species *Carpinus orientalis* and *Carpinus betulus* in Iran.

Materials and Methods

Pollen materials were obtained from their natural habitats and herbarium specimens of Nowshahr Botanical Garden (HNBG). The localities and numbers of voucher specimens are presented in Table 1. Fertile Anthers were processed according to the standard acetolysis method (Erdtman, 1943). For SEM observations, the pollen grains from mature anthers were mounted using a fine needle on aluminum stubs with double sticky tape. Prepared stubs were sputter coated with gold in 2-6 minutes (LEO 440i, England). After coating, the specimens were observed with a scanning electron microscope at 15-25 KV voltages. Measurements of pollen grains were taken from the SEM micrographs. The average of polar and equatorial axes was based on the measurements of 10-15 pollen grains. The pollen morphological terminology follows that by Punt et al. (2007).

All SEM photomicrographs were taken at the laboratory of Plant Cell Biology, Science and Research Branch, Islamic Azad University, Tehran.

Results

The size of pollen grains is shown in table 2. The smallest pollen is found in *C. betulus* var. *parva*, the length of polar axis $24.95 \pm 0.18 \mu\text{m}$ and equatorial diameter $23.15 \pm 0.13 \mu\text{m}$. The largest pollen belongs

Table 2. Summary of pollen morphological data of different taxa of *Carpinus* species; polar length (P), equatorial width (E), thickness of exine (L), aperture dimensions (A), density of sculptural elements (S). Measurements in μm ; density of sculptural elements in number per 100 μm^2 .

Characteristics Taxa	P		E		P/E	L	A	S
	Mean \pm SD Min - Max		Mean \pm SD Min - Max					
<i>C. betulus</i> var. <i>betulus</i>	32.63 \pm 2.59 30.12-35.6		27.81 \pm 0.72 26.34-30.02		1.2	1.53	3.32 \times 1.86	450
<i>C. betulus</i> var. <i>parva</i>	24.95 \pm 0.18 23-27.2		23.15 \pm 0.13 23-23.3		1.02	1.17	1.42 \times 1.2	200
<i>C. orientalis</i> subsp. <i>macrocarpa</i>	33.53 \pm 1.28 34.2-34.8		28.12 \pm 2.26 25.06-30		1.24	1.19	3.23 \times 1.29	450
<i>C. orientalis</i> subsp. <i>orientalis</i>	35.18 \pm 3.96 29.23-37.22		28.16 \pm 1.01 26.79-28.84		1.19	1.75	4.23 \times 2.93	500

to *C. orientalis* subsp. *macrocarpa*, the length of polar axis $33.53 \pm 1.28 \mu\text{m}$ and equatorial diameter $28.12 \pm 2.26 \mu\text{m}$. The smallest thickness of exine is found in *C. betulus* var. *parva* ($1.02 \mu\text{m}$) and the largest thickness of exine pollen belongs to *C. orientalis* subsp. *orientalis* ($1.75 \mu\text{m}$). The smallest aperture dimension is found in *C. betulus* var. *parva* ($1.42 \times 1.2 \mu\text{m}$) and the largest aperture dimension pollen belongs to *C. orientalis* subsp. *orientalis* ($4.23 \times 2.93 \mu\text{m}$). According to the classification of shape and ratio of P/E by Erdtman (1943), the shape of the most of the taxa examined is subprolate, except *C. betulus* var. *parva* which is prolate sphaeroideus (table 2). The shape of the pores is more or less circular, margins are distinctly smooth, with a very slight aspis formed by the thickening of the tectum. This aspis area was observed in all of the taxa examined in this research by SEM, not in LM and this could be due to desiccation. Surface sculpture of exine is irregularly cone-shaped and the apex is pointed (Fig. 2). Sculptural density varies between 200-500 elements per 100 μm^2

Discussion

This study shows some differences in pollen grains of Iranian *Carpinus* species based on number of the pores. Triporate and tetraporate types were seen in *C. betulus* var. *parva*. Tetraporate type was seen in *C. orientalis* subsp. *macrocarpa*, and pentaporate type in *C. betulus* var. *betulus* and *C. orientalis* subsp. *orientalis*. It also confirms the results from the previous research that *Carpinus* is generally triporate or tetraporate and pentaporate (Wodehouse, 1935; Punt et al., 2007; Takhtajan, 1980) and shows some new objectives for researchers.

Moreover, Chen studied pollen of six genera of *Betulaceae* that includes *Carpinus pubescens* Burkill, *C. tschonoskii* Maxim, *C. laxiflora* (Siebold & Zucc.) Blume and *C. japonica* Blume and verified that

Carpinus species has 3 pores. The current results were not in favour of Iranian *Carpinus* species, in the order of different size of studied *Carpinus* species. The pollen of the Iranian *Carpinus* species is a little bigger than Chinese *Carpinus* species (Chen 1991).

The main shape is subprolate as can be seen in all of the Iranian *Carpinus* species except *C. betulus* var. *parva*. The size of pollen and thickness of exine are nearly confirmed with Blackmore et al., (2003) and Punt et al., (2007), but the P/E ratio in their research and Chen's (1991) research were suboblate less often oblate, in this research pollen shape were subprolate or prolate-spheroidal.

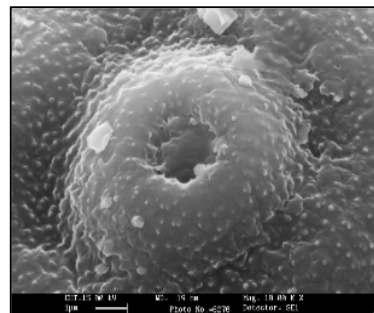
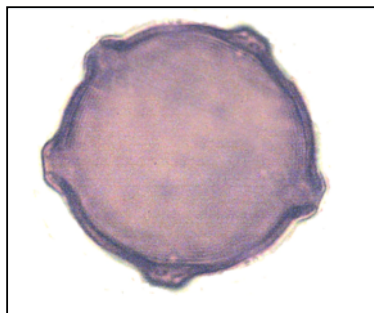
All species show a sunken area around the pore in SEM (not in LM) and apertures are simple and prominent. The ornamentation is variable and irregularly arranged. The density of sculptural elements is variable (200-500 per 100 μm^2). These results are a kind of pollen description in Iranian *Carpinus* species and they also indicated that main characteristics of pollen are pores, density of sculptural elements and thickness of exine.

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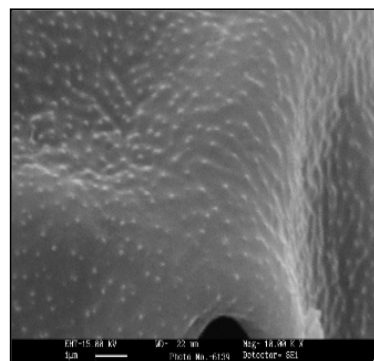
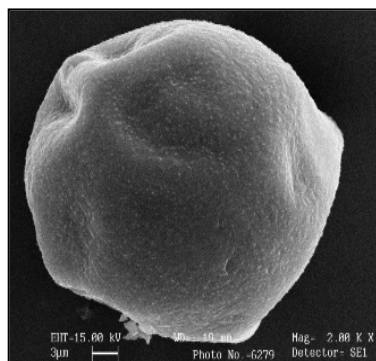
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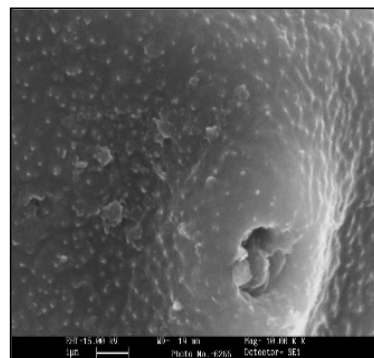
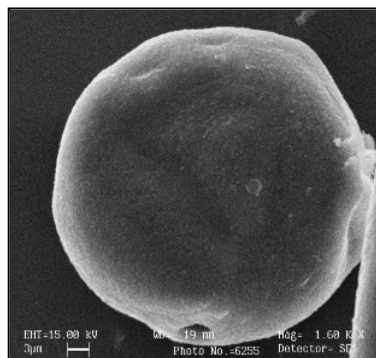
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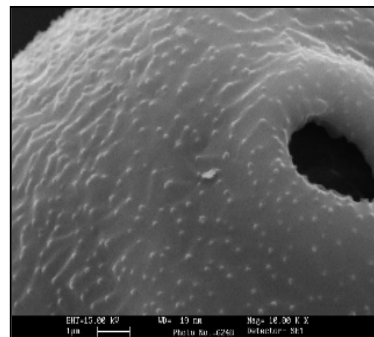
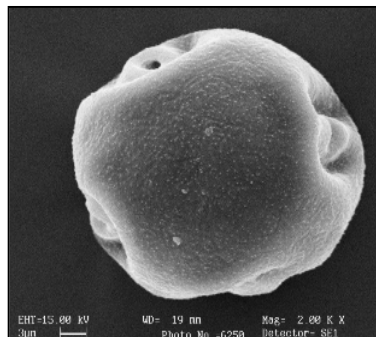
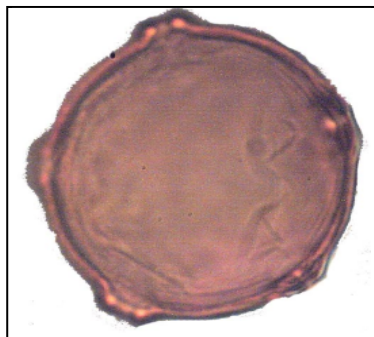
Carpinus betulus var. *betulus*



Carpinus betulus var. *parva*



Carpinus orientalis subsp. *macrocarpa*



Carpinus orientalis subsp. *orientalis*

Fig. 1. Pollen grains of the taxa examined (left: light microscopy, center and right: electron microscopy).

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