POLLEN MORPHOLOGY OF ARTEMISIA L. (ASTERACEAE) IN IRAN

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The pollen morphology of 26 species of *Artemisia* L. from 33 species that grow in Iran was investigated using light microscopy and scanning electron microscopy (SEM). Based on exine ornamentation observed under SEM, two types of pollen grains were recognized: type I, Exine surface have dense acute spinule or, Type II, exine surface have a sparse density of spinule. Pictures of all species and characteristics of pollen grain structure are presented.

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Key words. Pollen grain*, Artemisia*, SEM, acetolysis, Iran.

مطالعه ساختار دانه گرده جنس Artemisia L. (Asteraceae) در ایران

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ریخت شناسی دانه گرده ۲۶ گونه از جنس *Artemisia* L. (درمنه) از ۳۳ گونه درمنه ای که در ایران می رویند با استفاده از میکروسکوپ نوری(LM) و میکروسکوپ الکترونی (SEM) بررسی شد. بر اساس مطالعات تزئینات اگزین بوسیله میکروسکوپ الکترونی اسکنینگ، دو تیپ دانه گرده تشخیص داده می شود: در نوع I سطح اگزین دارای خارچه های نوک تیز ومتراکم است و در نوع II سطح اگزین دارای تراکم کمی از خارچه ها می باشد. تصاویر تمام گونه ها و مشخصات ساختار داخلی دانه های گرده ارائه شده است.

INTRODUCTION

The genus *Artemisia* L. is the largest genus of tribe *Anthemideae* (Asteraceae). *Artemisia* is mostly a perennial herb and rarely annual. They often occur as the dominant type in some plant communities as in steppe, subalpine steppe, shruby steppe, semidesert and desert steppe. They also occur in some coastal plains or ranges in lower diversity, distributed mainly on uncultivated hillsides (Wang 2004). The genus have over 400 species (Wright 2001) distributed primarily in the Asia, Europe, northern America and even south Africa. The origin and main distribution place of the genus is in Asia and then Europe (Heywood 1977). The species of the genus are distributed in Irano-Turanian and Hyrcanian regions and are a principal constituent of steppes. *Artemisia aucheri* and *A. sieberi* forms the dominant vegetation of steppes and semi steppes in the main part of Irano-Turanin region (Mozaffarian 1988).

The genus taxonomically is extremely complicated and difficult. These 400 species recognized by different authors have a great array of forms, hybridization simply occurs, and overlapping of features and morphologic polymorphisms are commonly seen.

The first classification of this genus was given by Besser (1829), who divided the genus into four sections based on fundamental differences in floral structure. Some taxonomists have elevated Besser's sections to the subgeneric level (Kelsey and Shafizadeh 1979; Poljakov 1961) and reduced the number of subgenera to three.

Historically, capitular and reproductive morphologies have been the diagnostic characters for the recognition of three subgenera in *Artemisia* L.. Poljakov (1961) in Flora of Russia, and Podlech (1986) in Flora Iranica had accepted these subgenera based on capitular and reproductive morphology: subgen. *Artemisia* L. (disciform, heterogamous capitula, with pistillate ray florets, and perfect fertile disk florets); subgen. *Dracunculus* (Besser) Rydb. (disciform, heterogamous capitula with pistillate ray florets and staminate disk florets); and subgen. *Seriphidium* (Besser) Rouy (discoid, homogamous capitula with perfect, fertile disk florets).

In previous studies (Skvarla et al. 1977; Edward 1994) two pollen structural types have been identified in *Asteraceae*: anthemoid and caveate. "anthemoid"

was first applied to pollen grains of *Anthemideae* (Skvarla et al. 1977). Anthemoid pollen grains have basal columellae rising from the foot layer, and one to several levels of shorter columellae and internal tecta above the basal columellae. Caveate grains lack basal columellae, having instead an internal cavity.

Palynological study has also confirmed the existence of two pollen morphological patterns in the subtribe *Artemisiineae* (*Anthemideae*) which is an important taxonomic character in the subtribe: *Artemisia* L. and its allied and segregate genera, i.e. one with short spinules (*Artemisia* L. type) and the other with long spines (*Anthemis* Nobilis type). It is thus suggested that the plants with short spinules evolve from ones with long spines based on the order of their occurrences in the geological past (Martín et al. 2001, 2003).

Wodehose (1935) on the basis of the size of the spine vestiges, had grouped the species of *Artemisia* into four different palynological classes as follows: (1) spine vestiges conspicuous; (2) spine vestiges minute but still quite large enough to be seen with certainly under favorable conditions; (3) spine vestiges represented only by a vanishing trace or even entirely absent in some grains; (4) spine vestiges generally entirely absent, though a trace of them may occasionally be seen on some grains. Generally pollen grains are prolate to perprolate, 3-zonocolpate type with smooth inner surface of furrows, in polar view triangular–trilobed, exine thick and columellae unbranched shortening from equatorial plane toward polar plane.

In our study, pollen grains of 26 species belonging to three subgenera are examined using light microscope and scanning electron microscope.

MATHERIALS AND METHODS

The pollen materials were obtained from herbarium specimens of Central Herbarium of Tehran University (TUH). Voucher specimens are listed in table 1. For scanning electron microscopy, pollen grains of mature capitula were transferred to stubs and coated with gold for 5-6 minutes. For preparing light microscope slides the acetolysis mixture (nine parts of acetic anhydride with one of sulphuric acid), purred into the pollen and heated for 15 to 20 minutes in boiling water (Erdtman 1969). The color of the mixture would become dark brown due to the presence of essential oils and floral fragments. The mixture should be centrifuged to sediment the grains. Three more centrifugation with water should be carried out to wash the pollen grains. Then the pollen grains are transferred to slides mounted with glycerin jelly. The measurements were carried out using light microscopy and based on at least 20 readings for each specimen.

Photos of the pollen grains were taken by a LEO 440i Scaning Electron Microscope (SEM) and using an Olympus Light Microscope for LM.

RESULTS

Based on LM and SEM observations, pollen grains are usually radially symmetrical, isopolar, normally tricolporate and 22.2 to 34.7 μm in diameter. Furrows are long and tapering toward both ends, their membranes are smooth or spinose, provided with a germinal aperture. Exine is thick and coarsely granular. Spines are small. Grains generally appear rounded triangular in polar view, with the pores bulging out on the three sides; the exine is seen to be thickest in the middle of the lumen, tapering in thickness gradually. Perhaps the most noteworthy feature of *Artemisia* pollen grains is the minuteness of their spines. This character is variable among the species and has taxonomic value. The 26 species examined showed diversity in pollen data, as given in table 2.

1. Size

The size of pollen grains are different, from small to medium. The smallest grains (*A. scoparia, A. turanica*) have a polar axis of 22.26 and 22.57 μm and an equatorial axis of 12.8 and 13.16 μm (Figs. 12,17). The largest grains (*A. Absinthium, A. vulgaris*) have a polar axis of 34.68 and 32.84 μm and an equatorial axis of 19.58 and 17.25 μm (Figs. 1,9).

2. Shape

The shape classes of pollen grains were determined according to the P/E ratio. In all species examined, the outline of pollen grains seen in polar view is circular, triangular and equatorial with an elliptic outline. Two distinct pollen shape classes could be recognized: prolate (P/E range: 1.46-1.91) and perprolate (P/E: 2.06). There was a small variation in shapes.

3. Aperture

The pollen apertures of all species examined is normally tricolporate. The colpi are usually wide at the equator and narrow near the poles (Fig. 1). The colpus membrane is often covered with granular elements or the colpi are narrow, smooth, and sunken. The colpi are not fused at the poles. Apertures are distinct with thick margins and are circular or elliptical (Figs. 8, 10, 17, 24).

4. Exine ornamentation

Exine ornamentation is echinate, as observed by SEM and LM (Figs.27-34). Echinate ornamentation, with

microgranula, is either present or absent on the surface. Based on exine ornamentation observed under SEM, two types of pollen grains were recognized: type I, Exine surface may have dense acute spinules such as: *A. fragrans*, *A. turanica* and *A. turcomanica* from Homogamous group (Figs. 17, 20, 25) and species as *A. biennis*, *A. persica* and *A. absinthium* from Heterogamous group (Figs. 3, 8, 9). Type II, exine surface may have a sparse density of spinules such as: *A. sieberi*, *A. spicigera* and *A. santolina* from Homogamous group (Figs.16, 21, 26) and species as *A. incana*, *A. scoparia* and *A. hausskenechtii* from Heterogamous group (Figs. 7, 10, 12).

5. Nexine and Sexine (Wall Thickness)

Nexine thickness in all the species is almost the same and 1 μm, but sexine thickness varies. The thickest sexine belongs to *A. marschaliana*, *A. vulgaris* and *A. austriaca* with the thickness of 3 to 3.1 μm. The thinnest sexine belongs to *A. diffusa* (S=1.13 μm) and *A. scoparia* (S=1.46 μm).

DISCUSSION

The species investigated in the present study covers all subgenera of *Artemisia.* The pollen of this genus is homologous. The shape of the pollen grains in *Artemisia* is prolate to perprolate in the equatorial view and is three-lobed circular in the polar view. Pollen grains are usually radially symmetrical, isopolar, tricolporate, with echinate sculpture. This is in agreement with previous work by Wodehose (1935) who divided the pollen type of the genus into 4 classes based on the size of the spine vestiges. *Artemisia* pollen grains in Iran belong to the first class (i.e. with conspicuous spine vestiges). Pollen characteristics among the species are similar. The characteristics of pollen grains are not of much taxonomic significance in *Artemisia.* The division of the genus into three subgenera on the basis of floral structure is not achieved by palynological data, because the pollen morphology of the species investigated is very similar in size, shape, sculpture, and colpus. These data are not of much diagnostic value to separate all the taxa of the genus, but can be useful in some species. For example in the subgenus *Artemisia*, division of *A. haussknechtii* and *A. austriaca* is based on the size of the plant. Palynological data support the distinction of the two species based on the density of spines on the exine surface. *A. austriaca* has a denser echinate surface than *A. haussknechtii*. Also distinguishing *A. Absinthium* and *A. incana* is based on the shape of inflorescence that is in agreement with palynological data, *A. absinthium* surface is denser than *A. incana*. But distinguishing other species like *A. biennis* and *A. annua* or *A. persica* and *A. chamaemelifolia* is not in agreement with pollen data. In the subgenus *Dracunculus, A. tschernieviana* is a woody plant with a denser echinate surface than *A. scoparia* witch is mostly a biennial herb. In the subgenus *Serephidium*, leaf size is less than 2 cm or longer to 8 cm. Species with polar length of pollen grains less than 25 μm have a small leaf (i.e. less than 2 cm) but species with polar length of pollen grains more than 25 μm have longer leaves (i.e. more than 2 cm). The only exception is *A. deserti* (Fig. 15) with polar length of 30.39 μm, its leaves are shorter than 2 cm.

Our results, correlated to other data such as morphology, allow us to conclude that the genus *Artemisia* as currently defined is a very heterogeneous group.

REFERENCES

Edward, L. 1994: Plesiomorphic and apomorphic pollen structure characteristics of Anthemideae (Asteroideae: Asteraceae). -American Journal of Botany 81 (5): 648-657.

Erdtman, G. 1969: Handbook of Palynology: morphology-taxonomy-ecology. -Munksgaard.

Garcia, S. 2004: Variation of DNA amount in 47 populations of the subtribe Artemisiinae and related taxa (Asteraceae, Anthemideae): karyological, ecological, and systematic implications. -Genome 47: 1004–1014.

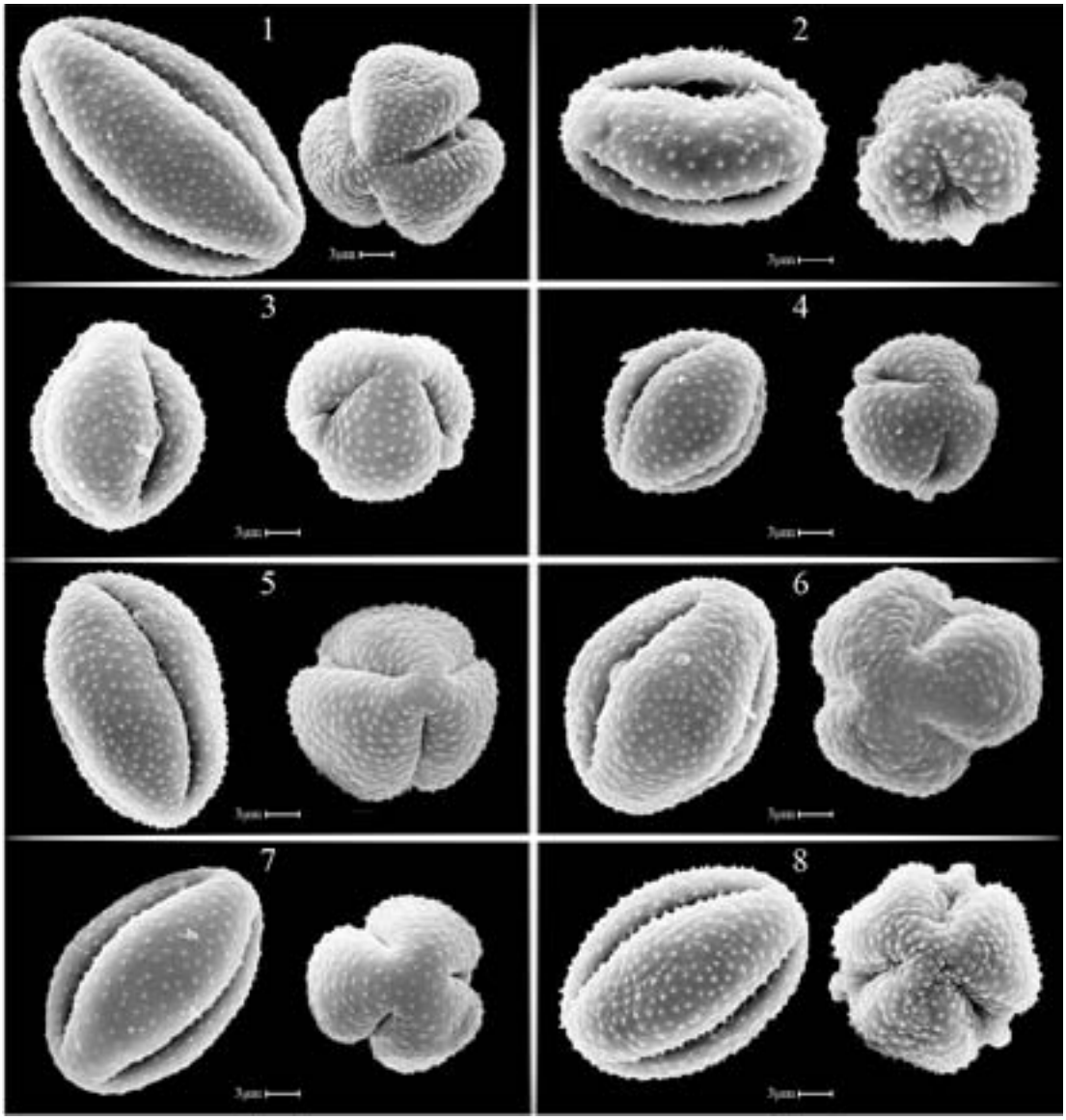
Heywood V.H. 1977: The Biology and chemistry of the Compositae, -Academic press, London, vol I, chap I, pp. 1-19.

Kelsey, R. G. & Shafizadeh, F. 1979: Sesquiterpene lactones and systematics of the genus Artemisia. -Phytochemistry, 18 (10): 1591-1611.

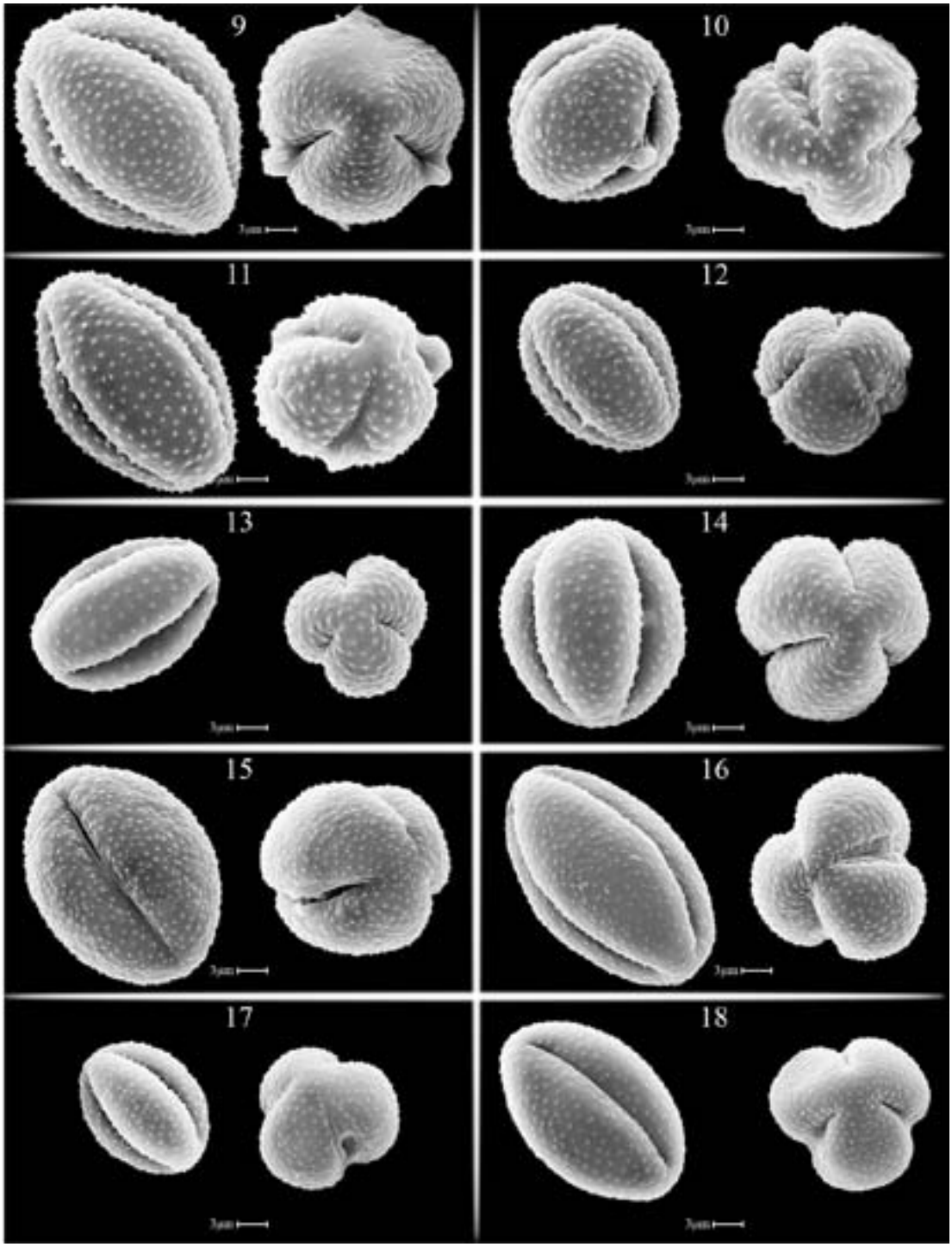
Kornkven, A. B. 1998: Phylogenetic analysis of Artemisia section Tridentatae (Asteraceae) based on sequences from the internal transcribed spacers (ITS) of nuclear ribosomal DNA. -American Journal of Botany 85(12): 1787–1795.

Martín, J., Torrell, M., & Vallès, J. 2001: Palynological features as a systematic marker in Artemisia L., and related genera (Asteraceae, Anthemideae). -Plant Biol. 3: 372–378.

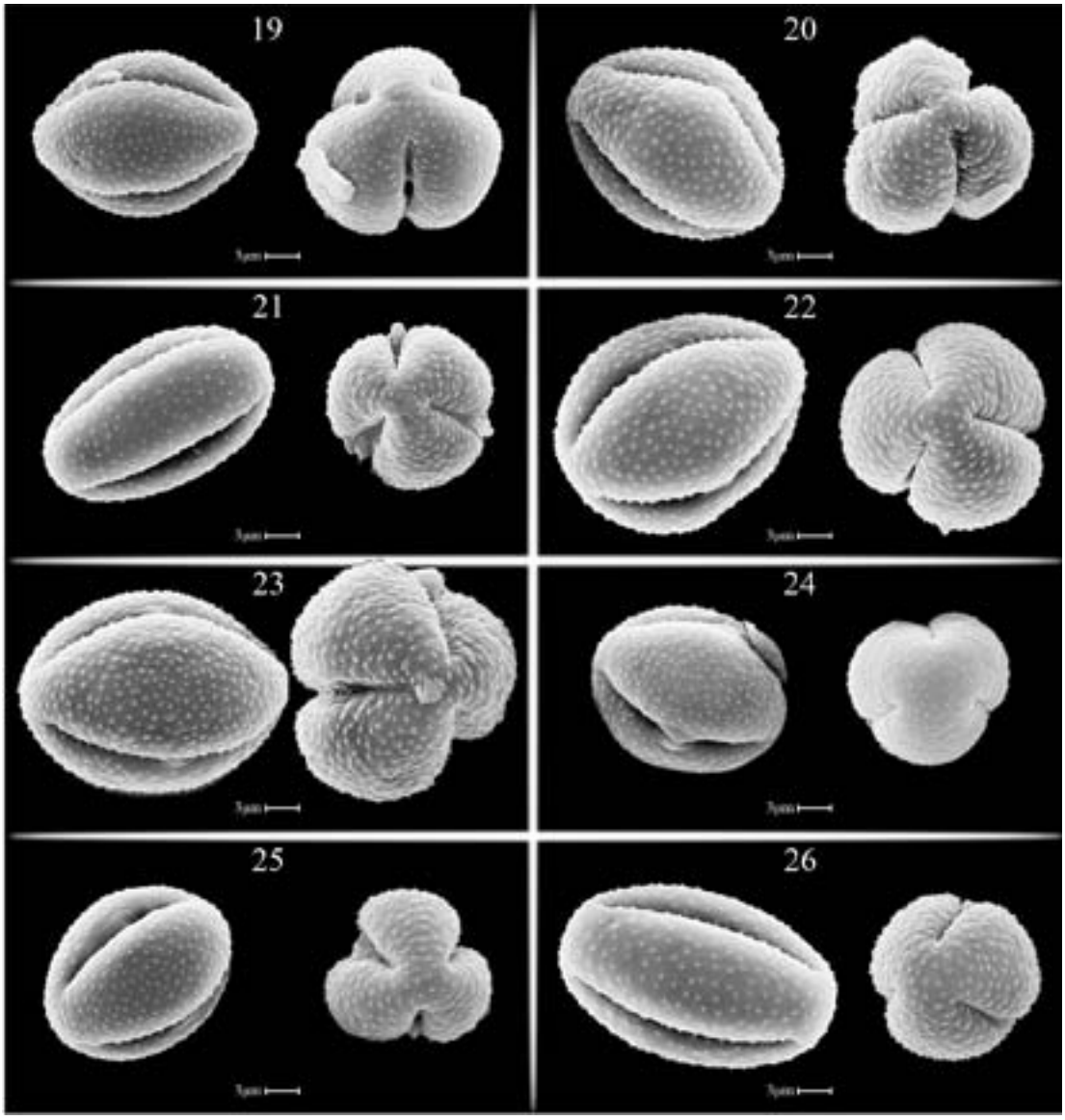
Martín, J., Torrell, M., Korobkov, A.A., and Vallès, J. 2003: Palynological features as a systematic marker in Artemisia L., and related genera (Asteraceae, Anthemideae), II: implications for subtribe Artemisiinae delimitation. -Plant Biol. 5: 85–93.



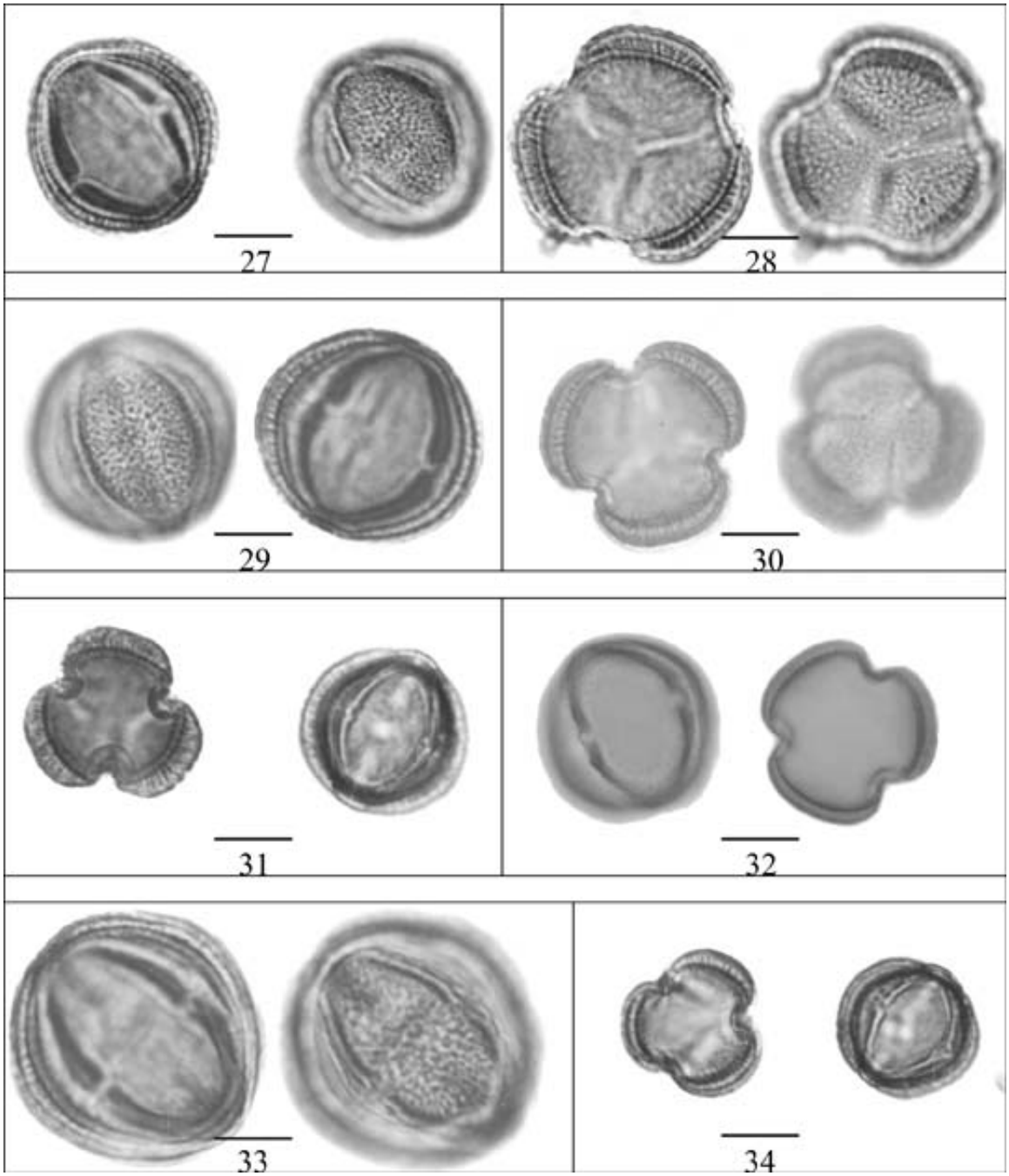
Figs. 1–8. Shape of pollen grains under a scanning electron microscope (SEM), polar views right and equatorial views left. 1. *Artemisia vulgaris*, 2. *A. chamaemelifolia*, 3. *A. biennis*, 4. *A. annua*, 5. *A. splendens*, 6. *A. austriaca*, 7. *A. haussknechtii,* 8. *A. persica.* Bar = 3 μm.



Figs. 9–18. Shape of pollen grains under a scanning electron microscope (SEM), polar views right and equatorial views left. 9. *Artemisia absinthium*, 10. *A. incana*, 11. *A. armeniaca*, 12. *A. scoparia*, 13. *A. tschernieviana*, 14. *A. marschaliana*, 15. *A. deserti,* 16. *A. santolina,* 17. *A. turanica,* 18. *A. diffusa.* Bar = 3 µm.



Figs. 19-26. Shape of pollen grains under a scanning electron microscope (SEM), polar views right and equatorial views left. 19. *A. kopetdaghensis*, 20. *A. turcomanica*, 21. *A. sieberi*, 22. *A. olivieriana*, 23. *A.* sp., 24. *A. aucheri.* 25. *A. fragrans*, 26. *A. spicigera.* Bar = 3 μm.



Figs. 27-34. Shape of pollen grains under a light microscope (LM) after acetolysis. 27. *Artemisia absintium* equatorial view, 28. *A. absintium* polar view, 29. *A. vulgaris* equatorial view, 30. *A. vulgaris* polar view, 31. *A. incana*, 32. *A. splendens*, 33. *A. santolina* 34. *A. turanica.* Bar = 10 µm.

Table 1. Specimens examined (voucher specimens from which pollen grains was sampled have been deposited at the Central Herbarium of University of Tehran.)

Subgen. *Artemisia*

Artemisia vulgaris L., Gilan: Bandar-e Anzali, alt. 25m, date: 21.9.1988, collector V. Mozaffarian, no. 7135.

A. chamaemelifolia Vill., date: 24.6.2001, alt. 2150m, col. B. Eslami, no. 33527.

A. biennis Willd., Mazandaran: Road of HAraz: Sangdeh, alt. 1800m, date: 20.10.1995,vol. Ghahreman & Attar, no. 20233.

A. annua L., Mazandaran: Road of Haraz: Sngdeh, alt. 1800m, date: 20.10.1995, col. Ghahreman & Attar, no. 20232.

A. splendens Willd., Urumieh, Silvana, Mavana, Kuh-e Khizan, alt. 2600-3000m, date: 6.7.1991, col. V. Mozaffarian, no. 69904.

A. austriaca Jacq., Azarbaiejan: ca. 17km from Ahar to Tabriz, alt. 1400m, date: 30.8.1993, col. A. Ghahreman & V. Mozaffarian, no.17610.

A. haussknechtii Boiss., Khorramabad: Sefid-Kuh, alt. 2500m, date: 22.7.1999, col. Veiskarami, no. 23690.

A. persica Boiss., Kerman: 40 km to Baft, south of Gugher, alt. 2880m, date: 28.5.2002, col. Ghahreman, Attar & Mehdigholi, no. 28604.

A. absinthium L., Firuzkuh, pass. Kadook, alt. 2000m, date: 5.12.2001 ,col. B. Eslami, no. 33538.

A. incana (L.) Druce, Zanjan to Bijar, alt. 1840m, date: 20.10.1987, col. V. Mozaffarian no. 59471.

A. armeniaca Sam. Azarbaiejan: Arasbaran protected area Kuh-e Kallan, alt. 2500m, date: 26.8.1993, coll. A. Ghahreman & V. Mozaffarian, no. 17572.

Subgen. *Dracunculus*

A. tschernieviana Besser, Mazandaran: Behshahr, Zaghmarz, alt. see level, date: 28.10.1987, col. V. Mozaffarian, no. 59623.

A. scoparia Waldst. & Kit., Gorgan: Bandar-e Turkman, Ashuradeh, alt. see level, date: 29.10.1987, col. V. Mozaffarian, no. 59640.

A. marschalina, Azarbaiejan: ca. 20 km from Ardabil to Khalkhal, Hir to Shibli to Gheshlagh, alt. 2400m, date: 30.9.1991, col. V. Mozaffarian, no. 70131.

Subgen. *Serephidium*

A. deserti Ktasch., Tehran: Firuzkuh. Mahmoudabad, date: 11.10.1990, col. A. Ghahreman & V. Mozaffarian, no. 9833

A. santolina Schrenk., Semnan: Shahrud, 20 km from Mayamay to Jilan (Chehel Dokhtar), alt. 1100m, date: 27.10.1987, col. V. Mozaffarian, no. 59605.

A. turanica Krasch., Khorassan: 17 km from Shirvan to Sovaldi (12 km from Ziarat to Sovaldi), alt. 1380m, date: 31.10.1987, col. V. Mozaffarian, no. 59698.

A. diffusa Krasch. ex Poljak., Khorassan: 26 km from Bojnourd to Gilan, date: 31.10.1987, col. V. Mozaffarian, no. 59684.

A. kopetdaghensis Krasch., M. Pop. & Lincz. ex Poljak., Khorassan: N. of Shirvan, Sovaldi, alt. 1200m, date: 31.10.1987, col. V. Mozaffarian, no. 59694.

A. turcomanica Grand., Khorassan: 80km to Bojnurd from Gorgan, Zamansufi, alt. 1500m, date: 30.10.1987, col. V. Mozaffarian, no. 59665.

A. sieberi Besser, Semnan: 20 km from Shahrud to Mayamay, alt. 1390m, date: 26.10.1987, col. V. Mozaffarian, no. 59575.

A. olivieriana J.Gay ex DC., Semnan: road from Semnan to Firuzkuh, alt. 1900m, date: 27.9.1994, col. A. Ghahreman & V. Mozaffarian, no. 18113.

A. sp., Semnan: 85 km to Azadshahr from Shahroud, alt. 700m, date: 27.10.1987, col. V. Mozaffarian, no. 59616.

A. aucheri Boiss., Khorassan: Birjand: Razg, alt. 1811m, date: 31.10.1997, col. Aliyabadi, no. 22133.

A. fragrans Willd., Tehran: Dmavand, Jaban, Garamasard, Gavij, date: 11.10.1990, col. A. Ghareman & V. Mozaffarian, no. 9839.

A. spicigera C. Koch., Tehran: Damavand-Rineh, col. V. Mozaffarian, no. 11374.

Table 2. Pollen morphology data for *Artemisia.*

	P† (µm)	E† (µm)	P/E	PS	Wall thickness (µm)
Subgen. *Artemisia*					
Artemisia vulgaris	31.5-35(32.84)	15.7-20.3(17.25)	1.9	prolate	4.01
A. chamaemelifolia	22.7-26.2(24.22)	14-17.5(16.48)	1.46	prolate	3.22
A. biennis	21-28(25.07)	10.5-15.7(14.56)	1.372	prolate	3.43
A. annua	20.3-24.5(23.01)	12.2-15.7(13.87)	1.65	prolate	2.94
A. splendens	29.7-34.3(32)	14-19.2(16.88)	1.89	prolate	3.24
A. austriaca	30.4-35(32.69)	14-21(17.11)	1.91	prolate	4
A. haussknechtii	22.7-28(24.69)	12.2-15.7(13.73)	1.79	prolate	2.82
A. persica	26.2-29.7(28.63)	14-17.5(15.59)	1.83	prolate	3.31
A. absinthium	31.5-40.2 (34.68)	17.5-22.7(19.58)	1.87	prolate	3.48
A. incana	23.8-29.7(25.9)	14-15.7(14.8)	1.75	prolate	3.24
A. armeniaca	26.2-28.7(27.5)	14-17.5(16.22)	1.69	prolate	3.38
Subgen. *Dracunculus*					
A. tschernieviana	23.8-29.7(27)	14-17.5(16.2)	1.6	prolate	3.57
A. scoparia	19.2-24.5(22.26)	11.2-15.7(12.8)	1.73	prolate	3.24
A. marschalina	22.7-29.7(26.7)	12.2-16.8(14.8)	1.8	prolate	4.1
Subgen. *Serephidium*					
A. deserti	28-33.2 (30.39)	15.7-21(18.46)	1.64	prolate	3.15
A. santolina	28.7-35(31.46)	14-17.5(15.22)	2.067	perprolate	3.42
A. turanica	19.2-24.5 (22.57)	10.5-14.7(13.16)	1.71	prolate	2.71
A. diffusa	22.7-28 (25.11)	12.2-15.7(14.1)	1.78	prolate	2.13
A. kopetdaghensis	26.2-31.5(28.07)	12.9-17.5(15.02)	1.86	prolate	3.13
A. turcomanica	23.8-31.5(27.4)	13.3-17.5(15.6)	1.81	prolate	3.81
A. Sieberi	21-26.2(23.92)	11.2-14(12.8)	1.86	prolate	2.65
A. olivieriana	28-31.5(29.89)	15.7-18.2(16.66)	1.61	prolate	3.26
A. sp.	28-35(30.92)	14-19.2(16.6)	1.86	prolate	3.67
A. aucheri	24.5-28(25.1)	14-17.5(15.36)	1.63	prolate	3.48
A. fragrans	26.2-29.7 (27.4)	15.7-14(14.7)	1.86	prolate	3.32
A. spicigera	21-29.7(26.61)	14-17.5(15.26)	1.73	prolate	2.9

E, equatorial axis; P, polar axis; PS, pollen shape; wall thickness includes: nexine (=1) + sexine. †, data are the range, with the means given in parentheses.

Moore, P.D. 1991: Pollen analysis. Pp. 62-82. -Blackwell Scientific Publications.

Mozaffarian, V. 1988: Botanical Study of Artemisia L. in Iran. Thesis for master degree of science, -Faculty of Science, Tehran University.

Nair, P.K. K. 1962: Pollen Grains of Indian Plants. -Bull. Natt. Bot. Gard. Lucknow. 63:1-33.

Naseri, H.R. 2004: Botanical and Ecological Studies of the Species of Artemisia L. in East Azarbaiejan Province, Thesis for master degree of science, -Faculty of Natural Resources. Tehran University.

Podlech, D. 1986: Artemisia in K. H. Rechinger, Flora Iranica, Compositae, Anthemideae VI, no.158: 159-223 , -Akademische Druck-U. Verlagsanstalt Graz-Austria.

Poljakov, P. P.1961: Artemisia. -*In* V. L. Komarov [ed.], Flora USSR, vol. 26, 425–631.

Skvarla, J. J., Turner, B. L., Patel, V. C. & Tomb, A. S., 1977: Pollen morphology in the Compositae and in morphologically related families. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.), The Biology and Chemistry of the Compositae. -Academic Press, London, pp. 141–248.

Wang, W. 2004: On the origin and development of Artemisia (Asteraceae) in the geological past. -Botanical Journal of the Linnean Society 145: 331–336.

Wodehose, R. P. 1935: Pollen grains; their structure, identification and significance in science and medicine. -McGraw-Hill Book Company. first edition.

Wright, C. W. 2001: Artemisia, Medicinal and Aromatic Plants-Industrial Profiles. Pp. 1-12. -Taylor and Francis, first edition.

Wu, D. 2005: Pollen morphology of Parnassia L. (Parnaciaceae) and its systematic implications. -Journal of Integrative Plant Biology 47 (1): 2-12.