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PALYNOLOGICAL STUDY OF THE GENUS *ARTEMISIA* (ASTERACEAE) AND ITS SYSTEMATIC IMPLICATIONS

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Abstract

In this study, using light and scanning electron microscopy the palynological study of 22 taxa of the genus *Artemisia* has been carried out. Pollen grains, of all the taxa observed, are tricolporate, represented by globular symmetry (3-lobed round in polar view and ellipsoid ball shaped in equatorial view) and marked by reduced spinules on their surfaces. The presence of spinules is a diagnostic character for *Artemisia* limb of tribe Anthemideae of family Asteraceae. Eight micromorphological characters (pollen shape, spinules arrangement, exine sculpture, spinules base, polar length, equatorial width, exine thickness and colpus width) of pollen grains of *Artemisia* have been identified and pooled in analysis. In the resulting clusters, 5 groups within *Artemisia* have been recognized. This indicates that pollen morphology is good taxonomic marker and can be utilized for infrageneric classification of genus *Artemisia*.

Introduction

Artemisia L. is a one of main genus of the tribe Anthemideae and also one of the largest genera of the family Asteraceae. It is an eminent wind pollinated cosmopolitan genus, chiefly found in temperate regions of mid to high latitudes of the northern hemisphere, settled in arid and semiarid climates areas and has only a small numbers representation in southern hemisphere (McArthur & Plummer, 1978; Valles & McArthur, 2001). Most of species of the genus have a great economic importance as medication, foodstuff, rummage, ornamentals or soil stabilizers in agitate habitats; some taxa are lethal or allergenic and some others are insidious weeds which can harmfully affects the harvests (Pareto, 1985; Tan *et al*., 1998; Hayat *et al*., 2009a). Many species in the genus are perennial; only just about 10 species are annuals or biannual (Valles *et al*., 2003). *Artemisia* is also recognized as pointer of steppe climate (Erdtman, 1952) and reasonable precipitation (El-Moslimany, 1990).

After different systematic reshufflings (Hooker, 1881; McArthur *et al*., 1981; Ling, 1982, 1991a, 1991b, 1995a, 1995b; Bremer, 1994; Kornkven *et al*., 1998; Torrell *et al*., 1999), the genus was alienated into 5 large assemblages which have been treated at sectional or subgeneric level; *Absinthium* (Tournefort) de Cand., *Artemisia* Tournefort (=*Abrotanum* Besser), *Dracunculus* Besser, *Seriphidium* Besser and *Tridantatae* (Rydb.) McArthur which is only restricted to North America. Ling (1991a, 1995b) segregated *Seriphidium* from *Artemisia* as a separate genus. Bremer (1994) agreeded this separation but Torrell *et al*., (1999) and Watson *et al*., (2002) once again integrated *Seriphidium* with *Artemisia*. But still a unambiguous depiction of natural classification within *Artemisia* has not been established.

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Investigation of pollen morphology of *Artemisia* has been started from the time of Wodehouse (1926). The following workers such as Monoszon (1948, 1950a, 1950b), Straka (1952), Stix (1960), Singh & Joshi (1969), Dimon (1971), Praglowski (1971), Korobkov (1981), Persson (1974), Valles *et al.*, (1987), Caramiello *et al*., (1987; 1989; 1990), Lodari *et al*., (1989), Vezey *et al*., (1994), Martin *et al*., (2001; 2003) and Jiang (2005) elaborated different perspectives of *Artemisia* pollen characteristics including the structural organization, size and shape of pollen, sculptural diversity in exine, aperture magnitudes etc., along with their taxonomic application.

Pollen grains of this genus are comparatively unique and straightforwardly identifiable and are marked by tiny spines or without spines (Bremer & Humphries, 1993). They are minute in size but bulky in amount (Jiang, 2005). Martin *et al*., (2001; 2003) carried out a palynological investigation on the tribe Anthemideae together with *Artemisia*, its allies and set apart genera and found that ornamentation with tiny spinules is value able taxonomic tool for *Artemisia* and its taxonomic relatives. It gives the impression that the pollen morphology of *Artemisia* has hang about unaffected through out its reported history, as pointed out by morphological association between fossils and modern pollen grains (Wang, 2004). Wang (2004) also point out that *Artemisia* type pollen with short spinules evolved from ones with long spines (*Anthemis* type) based on the order of their occurrences in the geological history.

This research work covers different palynological aspects of the pollen grains of the genus *Artemisia* species form Pakistan. In this study we mainly focused on the taxonomic utility of micromorphological characters of pollen for taxonomic application within the genus.

Materials and Methods

Pollen material used in this study has been obtained from herbarium specimens as well as freshly collected during the expeditions to various parts of Pakistan. Origin and detail of studied taxa is as such as was in our previous studies (Hayat *et al.,* 2009b, 2009c, 2010). The pollen grains were prepared for scanning electron microscopy (SEM) and light microscopy (LM) by the standard methods, described by Erdtman, (1952) and modified by Perveen *et al*., (1994), Perveen and Qaiser (2007; 2009; 2010) and Bibi *et al*., (2008). For SEM pollen grains were acetolysed and directly transferred to the sticky carbon disc on metal stub and coated with platinum in a sputtering chamber (K5 75x Sputter Coater). LEO Supra 55VP scanning electron microscope was operated at 5 kV, at Royal Botanical Garden, Edinburgh, Scotland, UK. For LM the pollen grains were mounted on glass slide in saffranin stained glycerin jelly and sealed with transparent nail varnish. Micromorphological observations of pollen grains were made with OLYMPUS/BX-51 light microscope at National Center of Excellence in Geology, University of Peshawar, Pakistan. The measurements of pollen are based on 25 to 30 readings from each specimen. Observations for polar diameter (P), equatorial diameter (E) and P/E ratio were taken in such a way that for each parameter, the arithmetic mean and standard deviation are given according to Erdtman, (1952) and Reitssma (1970). From observation of micromorphological characteristics of pollen in *Artemisia*, an original data matrix was produced (Table 3). This data matrix was subjected to cluster analysis by UPGMA method with EUCLIDEAN option, using the MVSP software version 3.13 (Kovach, 2007).

Results and Discussion

Quantitative observations by mean of LM are shown in Table 1. Figs. 1, 2 and 3 signify the pollen morphology in *Artemisia* by means of SEM. The micromorphological characters which show variations of taxonomic value are given in Table 1 and Fig. 4 illustrates the results of cluster analysis based on these features.

The polar and equatorial views of the *Artemisia* pollen can be easily recognized (Fig. 1 and 2). Fig. 3 shows the sculpture patrons of exine surface of *Artemisia* pollens which mainly marked by tiny spinules. These spinules are prominent in *A. dubia*, *A. amygdalina*, *A. vulgaris*, *A. japonica*, *desertorum* and *A. vestita* while other species showed degenerative tendency in their structure. Similarly the spinules densities notably vary among different taxa of the genus.

From LM and SEM observations it is clear that the shape of pollen grain is homogeneous throughout in the genus and verifies its monophyly as presented in molecular studies of Kornkven *et al*., (1998), Torrell *et al*., (1999) and Watson *et al*., (2002). Therefore, this study also supports the reunion of *Seriphidium* with *Artemisia*. The general features of *Artemisia* pollen show high concordance, which are recognized by the globular or the approximate symmetry, 3 lobed spheres in the equatorial view while ellipsoid in the polar view with tricolporate structure as reported by Jiang (2005). The exine of the pollen has a noticeable double layered structure of inner and outer layers with aggregated columella.

By LM and SEM, 8 micromorphological characters of pollen of *Artemisia* were selected for cluster analysis (Table 2 and 3). The results of cluster analysis (Fig. 4) suggest that pollen grains in 22 taxa from *Artemisia* can be classified in to 5 main groups. *S. kurramense* and *A. moorcroftiana* constitute group 1, *A. scoparia*, *A. stricta* and *A. desertorum* form group 2, group 3 include *A. siversiana*, *A. japonica*, *A. herba-alba*, *A. vestita* and *A. tournefortiana*, group 4 contains *A. absinthium*, *A. gmelinii*, *S. brevifolium*, *A. santolinifolia*, *A. rutifolia*, *A. vulgaris*, *S. turanicum* and *A. biennis* and group 5 comprises of *A. tangutica*, *A. roxburghiana*, *A. dubia* and *A. amygdalina*. Fig. 3 also compare this grouping with traditional classification based only on floral characters and has many objections as for example section *Artemisia* only differ from section *Absinthium* by a single character i.e., receptacle naked (*Artemisia*) or receptacle cover with long hairs (*Absinthium*) (Kaul & Bakshi, 1984).

Our results also proved the Jiang *et al*., (2005) hypothesis that it is very hard to distinguish different *Artemisia* species only by their pollen morphology. We also agreed with the conclusions and results of Martin *et al*., (2001 & 2002) that pollen morphology is a diagnostic feature for *Artemisia* and recognized as excellent taxonomic marker.

The tendency of pollen morphological evolution is to develop more and more degenerative features (Hayat *et al.*, 2009). Globular pollen shape, dense spinules arrangement, granular exine sculpture, broad spinule base, large pollen size, broad colpus width and thick exine, all are the plesiomorphic (primitive) traits of *Artemisia* pollen. In apomorphic (derived) condition these features have transformed to oblate pollen shape, lose spinules arrangement, sinuolate (without granules) exine sculpture, without prominent spinule base, small pollen volume, thin colpus and reduced exine thickness. One reason for this evolution is the pollination patterns i.e., form entomophily to anemophily. The other major cause of this evolution is change in climate patterns during the relocation from North Temperate Zone with high latitude high elevation to low latitude and low evaluation moist regions during the glacial epoch (Wang, 2004; Jiang *et al*., 2005).

Table 1. Pollen quantitative characteristics of the taxa studied.

Species	P	E	P/E	T	C
Section *Artemisia* Tournefort					
A. amygdalina	17.48 – 21.87 µm	17.69 – 23.06 µm		1.90 – 3.63 µm	13.63 – 18.81 µm
	X = 19.53 ± 1.20 µm	X = 20.98 ± 1.79 µm	0.93	X = 2.84 ± 0.53 µm	X = 16.25 ± 1.57 µm
A. biennis	18.55 – 23.05 µm	16.38 – 22.22 µm		1.80 – 3.32 µm	9.19 – 14.64 µm
	X = 20.88 ± 1.29 µm	X = 18.59 ± 2.04 µm	1.12	X = 2.56 ± 0.49 µm	X = 11.86 ± 1.63 µm
A. dubia	19.05 – 25.37 µm	17.72 – 22.42 µm		2.29 – 3.70 µm	10.84 – 17.07 µm
	X = 22.29 ± 1.90 µm	X = 19.62 ± 1.70 µm	1.14	X = 3.11 ± 0.36 µm	X = 13.93 ± 1.75 µm
A. gmelinii	18.98 – 24.77 µm	15.43 – 18.50 µm		2.62 – 3.66 µm	10.49 – 12.29 µm
	X = 21.38 ± 1.73 µm	X = 16.49 ± 1.14 µm	1.30	X = 3.08 ± 0.31 µm	X = 11.63 ± 0.54 µm
A. moorcroftiana	24.27 – 31.49 µm	18.91 – 24.09 µm		2.46 – 3.87 µm	12.96 – 18.84 µm
	X = 27.01 ± 2.16 µm	X = 21.70 ± 1.55 µm	1.24	X = 3.11 ± 0.44 µm	X = 16.04 ± 1.90 µm
A. roxburghiana	18.64 – 24.00 µm	18.51 – 21.73 µm		1.94 – 3.19 µm	12.22 – 15.88 µm
	X = 21.29 ± 1.70 µm	X = 20.25 ± 0.99 µm	1.05	X = 2.60 ± 0.43 µm	X = 13.78 ± 1.19 µm
A. rutifolia	18.37 – 22.35 µm	17.11 – 19.80 µm		2.47 – 3.62 µm	11.91 – 13.92 µm
	X = 20.33 ± 1.01 µm	X = 18.64 ± 0.94 µm	1.09	X = 3.04 ± 0.32 µm	X = 12.86 ± 0.72 µm
A. santolinifolia	19.21 – 24.64 µm	16.97 – 18.79 µm		2.12 – 3.17 µm	10.01 – 14.40 µm
	X = 21.35 ± 1.81 µm	X = 17.85 ± 0.61 µm	1.20	X = 2.71 ± 0.35 µm	X = 12.61 ± 1.36 µm
A. tournefortiana	15.92 – 21.42 µm	15.33 – 20.56 µm		1.25 – 3.03 µm	9.85 – 14.15 µm
	X = 18.66 ± 1.88 µm	X = 18.06 ± 1.82 µm	1.03	X = 2.26 ± 0.51 µm	X = 12.25 ± 1.47 µm
A. vestita	17.03 – 22.27 µm	15.31 – 20.96 µm		1.11 – 3.52 µm	8.91 – 15.43 µm
	X = 19.38 ± 1.52 µm	X = 18.09 ± 1.51 µm	1.07	X = 2.13 ± 0.67 µm	X = 11.81 ± 1.69 µm
A. vulgaris	18.63 – 22.82 µm	15.45 – 19.89 µm		1.66 – 3.24 µm	8.29 – 14.53 µm
	X = 21.04 ± 1.13 µm	X = 17.89 ± 1.35 µm	1.18	X = 2.57 ± 0.49 µm	X = 11.49 ± 1.91 µm

Table 1. (Cont'd.).

Section *Absinthum* (Mill.) DC																															
A. absinthium			17.72	–	23.07	µm			13.93	–	21.20	µm				2.23	–	3.52	µm			10.84	–	18.64	µm							
	X	=	20.47	±	1.994	µm	X	=	16.62	±	2.30	µm	1.23	X	=	3.03	±	0.45	µm	X	=	12.58	±	2.05	µm							
A. siversiana			16.26	–	20.48	µm			14.81	–	17.78	µm				1.62	–	2.49	µm			9.96	–	13.34	µm							
	X	=	17.88	±	1.58	µm	X	=	16.45	±	0.98	µm	1.09	X	=	2.07	±	0.28	µm	X	=	11.74	±	0.99	µm							
A. tangutica			18.61	–	22.92	µm			15.99	–	25.74	µm				1.90	–	2.92	µm			10.26	–	18.88	µm							
	X	=	20.62	±	1.43	µm	X	=	21.20	±	3.81	µm	0.97	X	=	2.47	±	0.38	µm	X	=	13.92	±	3.10	µm							
Section *Seriphidium* (Besser) Besser																															
A. herba-alba			16.27	–	21.49	µm			14.05	–	21.04	µm				1.50	–	3.17	µm			8.84	–	14.98	µm							
	X	=	19.02	±	1.44	µm	X	=	18.14	±	2.14	µm	1.05	X	=	2.42	±	0.50	µm	X	=	11.67	±	2.21	µm							
S. brevifolium			20.54	–	23.46	µm			16.62	–	19.20	µm				1.93	–	3.10	µm			11.99	–	15.20	µm							
	X	=	22.12	±	0.23	µm	X	=	17.96	±	0.74	µm	1.23	X	=	2.99	±	0.97	µm	X	=	13.32	±	1.21	µm							
S. turanicum			16.77	–	27.23	µm			16.86	–	19.88	µm				2.29	–	3.31	µm			11.46	–	12.00	µm							
	X	=	21.13	±	3.76	µm	X	=	18.18	±	1.27	µm	1.16	X	=	2.81	±	0.44	µm	X	=	11.68	±	0.24	µm							
S. kurramense			22.16	–	28.74	µm			17.89	–	22.83	µm				2.14	–	2.87	µm			10.11	–	15.54	µm							
	X	=	25.65	±	2.28	µm	X	=	19.99	±	1.37	µm	1.28	X	=	2.46	±	0.19	µm	X	=	12.42	±	1.81	µm							
Section *Dracunculus* Besser																															
A. desertorum			16.20	–	19.36	µm			12.53	–	17.15	µm				2.11	–	3.25	µm			7.24	–	12.58	µm							
	X	=	18.05	±	1.01	µm	X	=	15.16	±	1.46	µm	1.19	X	=	2.51	±	0.36	µm	X	=	10.17	±	1.90	µm							
A. japonica			16.36	–	20.72	µm			14.80	–	26.38	µm				2.36	–	3.66	µm			8.57	–	20.83	µm							
	X	=	18.97	±	1.24	µm	X	=	18.47	±	4.45	µm	1.03	X	=	3.12	±	0.46	µm	X	=	13.57	±	3.81	µm							
A. scoparia			14.78	–	19.69	µm			12.24	–	27.40	µm				2.29	–	3.52	µm			8.57	–	12.59	µm							
	X	=	16.39	±	1.48	µm	X	=	17.14	±	4.53	µm	0.96	X	=	2.93	±	0.33	µm	X	=	10.73	±	1.37	µm							
A. stricta			14.44	–	19.44	µm			13.83	–	15.83	µm				1.77	–	3.36	µm			6.90	–	11.74	µm							
	X	=	16.54	±	1.63	µm	X	=	14.84	±	0.65	µm	1.11	X	=	2.52	±	0.53	µm	X	=	9.67	±	1.33	µm							

P, polar axis; E, equatorial axis; X, arithmetic mean; P/E, sphericity; T, thickness of exine; C, colpus length

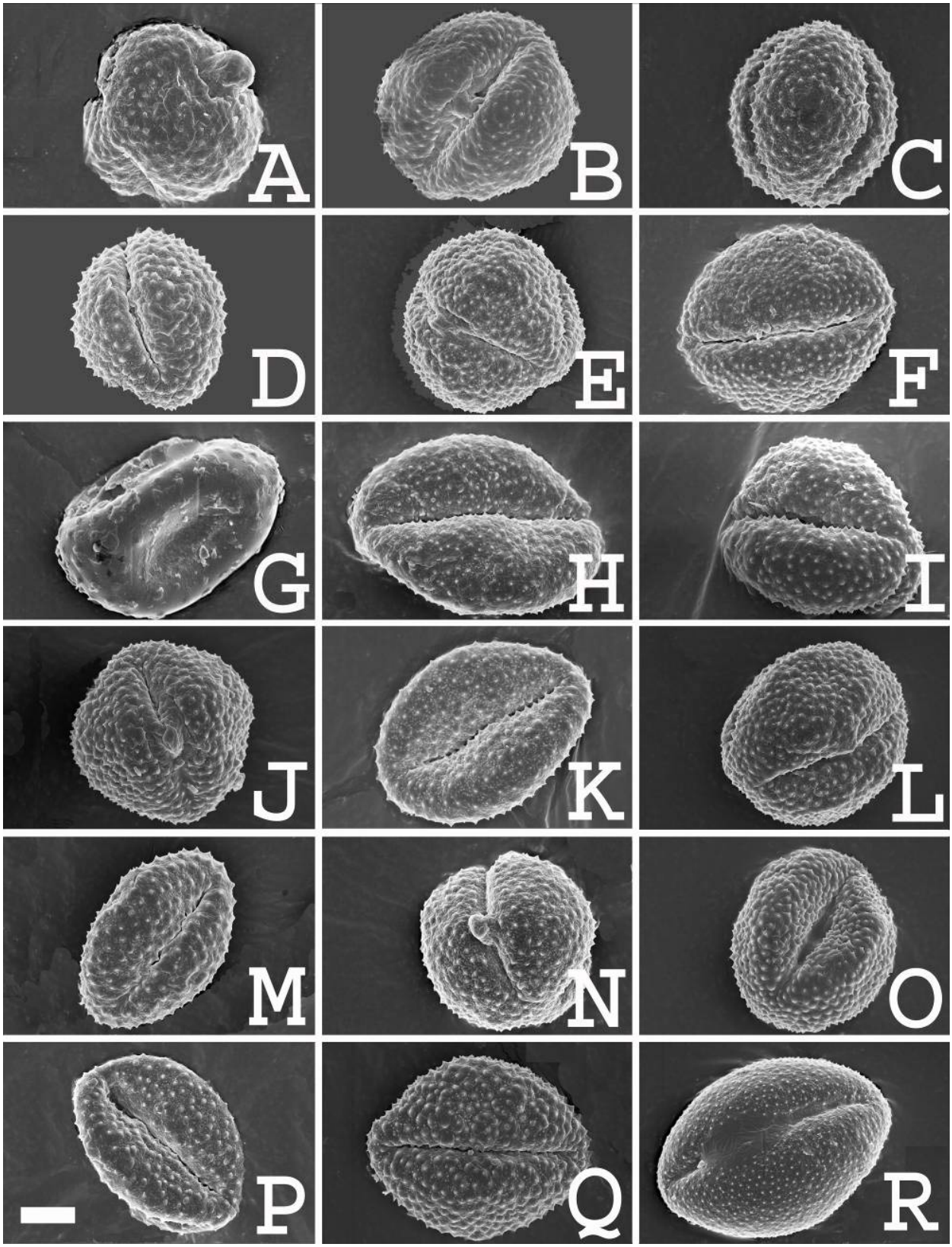

Fig. 1. Scanning electron micrographs showing the equatorial view of pollens of studied taxa: *A. amygdalina* (A), *A. herba-alba* (B), *A. desertorum* (C), *A. vestita* (D), *A. roxburghiana* (E), *A. moorcroftiana* (F), *A. maritima* (G), *S. turanicum* (H), *A. vulgaris* (I), *A. siversiana* (J), *A. japonica* (K), *A. dubia* (L), *A. biennis* (M), *A. tournefortiana* (N), *A. tangutica* (O), *A. rutifolia* (P), *A. absinthium* (Q), *S. kurramense* (R) . Scale bar = 2µm

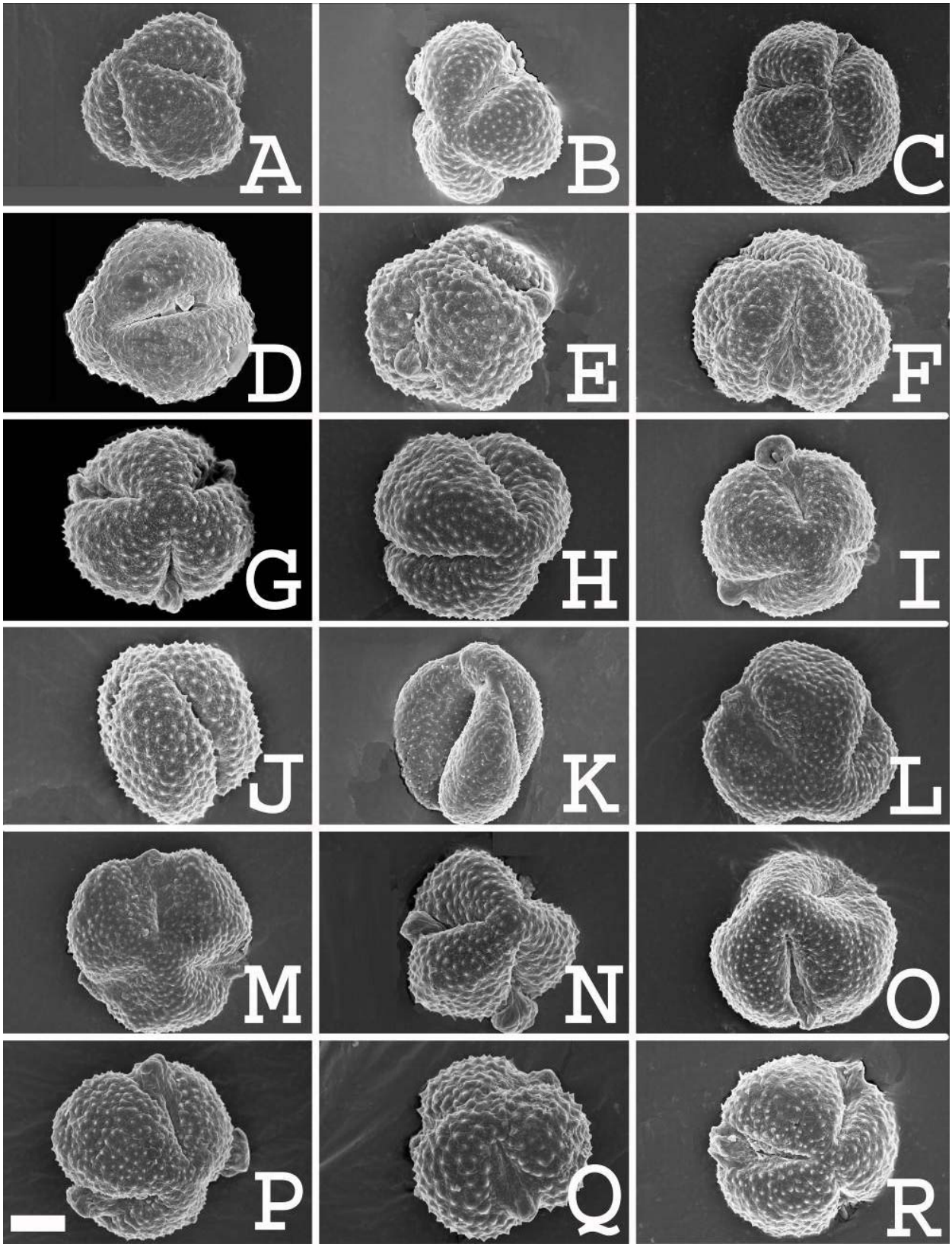

Fig. 2. Scanning electron micrographs showing the polar view of pollens of studied taxa: *A. desertorum* (A), *A. herba-alba* (B), *A. biennis* (C), *A. rutifolia* (D), *A. vestita* (E), *A. roxburghiana* (F), *A. moorcroftiana* (G), *A. dubia* (H), *A. japonica* (I), *A. tournefortiana* (J), *S. turanicum* (K), *A. tangutica* (L), *A. amygdalina* (M), *A. absinthium* (N), *S. kurramense* (O), *A. siversiana* (P), *A. stricta* (Q), *A. vulgaris* (R) . Scale bar = 2µm

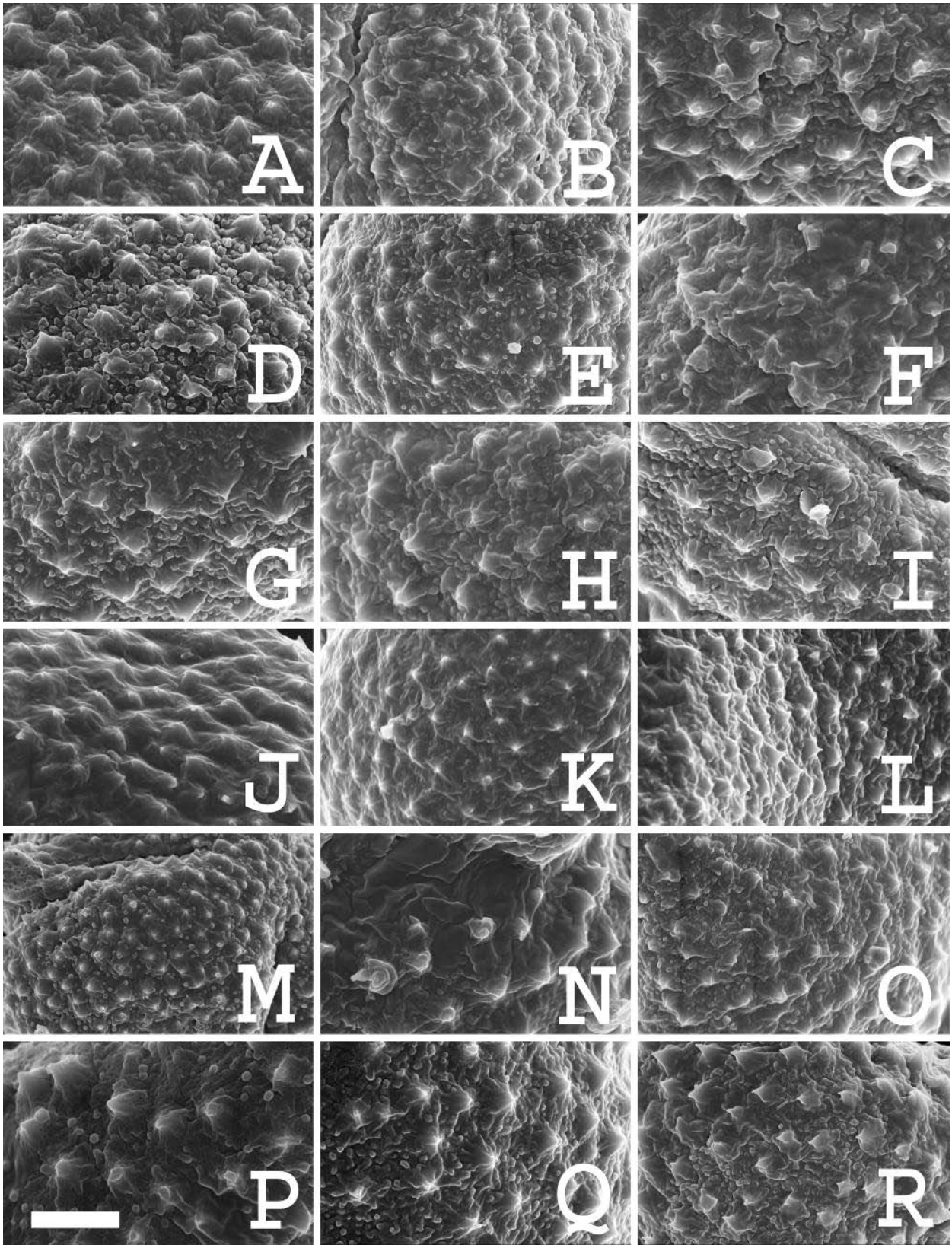

Fig. 3. Scanning electron micrographs showing the exine sculpture of studied taxa: *A. dubia* (A), *A. roxburghiana* (B), *A. moorcroftiana* (C), *A. vulgaris* (D), *A. biennis* (E), *S. turanicum* (F), *A. japonica* (G), *A. tournefortiana* (H), *A. amygdalina* (I), *A. tangutica* (J), *A. herba-alba* (K), *S. kurramense* (L), *A. siversiana* (M), *A. stricta* (N), *A. rutifolia* (O), *A. absinthium* (P), *A. desertorum* (Q), *A. vestita* (R) . Scale bar = 1µm

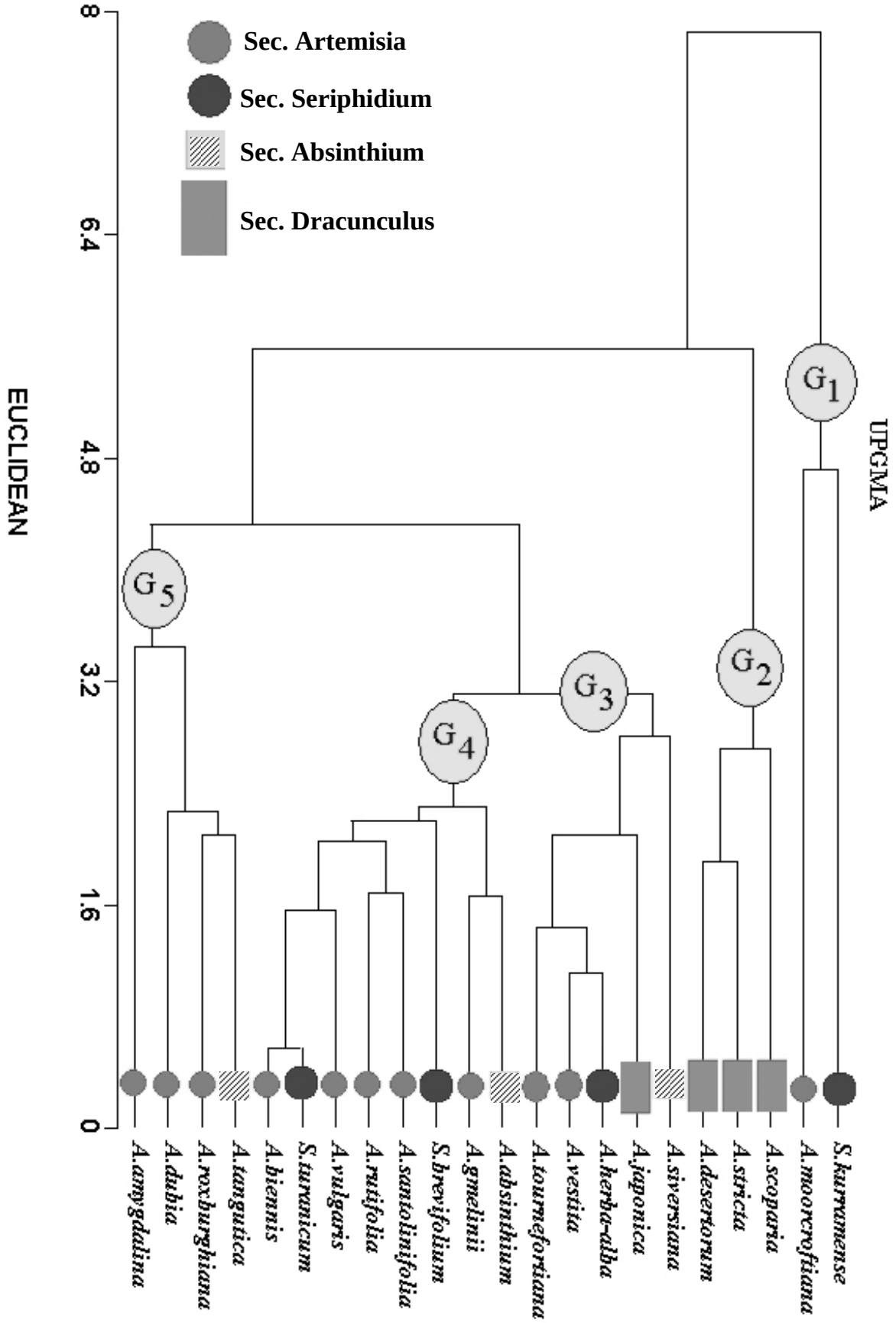

Fig. 4. Phenogram based on cluster analysis of micromorphological characters of pollen of *Artemisia*.

Table 2. *Artemisia* pollen characters and character states that are used for the cluster analysis. The number in brackets represents the codes of character states.

	Characters	Character states
1.	Pollen shape	Globular (0), Oblate (1)
2.	Spinules arrangement	Dense (0), Loose (1)
3.	Exine sculpture	Granular (0), Sinuolate (1)
4.	Spinules base	Stretching and outward extending (0) Normal (1)
5.	Polar length	Taken as such from Table 1
6.	Equatorial width	Taken as such from Table 1
7.	Exine thickness	Taken as such from Table 1
8.	Colpus width	Taken as such from Table 1

Table 3. Character state matrix used in cluster analysis of *Artemisia*. Characters and character states are described in Table 4.

Taxa	1	2	3	4	5	6	7	8
A. amygdalina	1	1	1	1	19.53	20.98	2.84	16.25
A. biennis	1	1	1	1	20.88	18.59	2.56	11.86
A. dubia	1	0	0	1	22.29	19.62	3.11	13.93
A. gmelinii	1	1	1	1	21.38	16.49	3.08	11.63
A. moorcroftiana	0	0	0	0	27.01	21.7	3.11	16.04
A. roxburghiana	0	1	1	1	21.29	20.25	2.6	13.78
A. rutifolia	0	1	0	0	20.33	18.64	3.04	12.86
A. santolinifolia	0	1	1	0	21.35	17.85	2.71	12.61
A. tournefortiana	0	1	1	1	18.66	18.06	2.26	12.25
A. vestita	0	1	0	0	19.38	18.09	2.13	11.81
A. vulgaris	1	0	1	0	21.04	17.89	2.57	11.49
A. absinthium	0	1	1	1	20.47	16.62	3.03	12.58
A. siversiana	0	0	1	0	17.88	16.45	2.07	11.74
A. tangutica	1	0	0	1	20.62	21.2	2.47	13.92
A. herba-alba	0	1	0	1	19.02	18.14	2.42	11.67
S. brevifolium	1	1	1	1	22.12	17.96	2.99	13.32
S. turanicum	1	1	1	1	21.13	18.18	2.81	11.68
S. kurramense	1	1	1	1	25.65	19.99	2.46	12.42
A. desertorum	1	1	0	1	18.05	15.16	2.51	10.17
A. japonica	0	1	0	1	18.97	18.47	3.12	13.57
A. scoparia	1	1	0	1	16.39	17.14	2.93	10.73
A. stricta	1	1	1	1	16.54	14.84	2.52	9.67

Conclusion

In the present study, it is recognized that micromorphological characters of pollen of *Artemisia* are taxonomically valuable traits at specific level in the genus, have great importance to add in argues of evolution and taxonomy of *Artemisia* within the genus. Sequence analysis of internally transcribed spacer and other investigations such as phytochemistry, karyology, phytogeography are collectively required to establish the subgeneric natural classification system of *Artemisia*.

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