African Journal of Biotechnology Vol. 8 (23), pp. 6561-6568, 1 December, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Phylogenetic analysis of *Artemisia* L. (Asteraceae) based on micromorphological traits of pollen grains

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Accepted 2 November, 2009

The phylogenetic relationships within the genus *Artemisia* have been very controversial. In order to recognize the systematic inference of pollen grains in this genus, the micromorphological characteristics of pollens from 22 taxa were examined by means of light microscopy (LM) and scanning electron microscopy (SEM). A phylogenetic analysis of *Artemisia* based on 9 micromorphological characters of pollens was conducted using Wagner parsimony method. In the resulting phylogenetic tree, relationships among different *Artemisia* species are shown. This study also presents the phylogenetic associations among 4 sections within the genus *Artemisia*. The reunion of genus *Seriphidum* with *Artemisia* was also confirmed.

Key words: Artemisia, Seriphidium, Anthemideae, Asteraceae, palynology, pollen morphology, taxonomy, systematics, phylogeny.

INTRODUCTION

Artemisia L. is the largest genus of the tribe Anthemideae and even one of the largest genera of the family Asteraceae. It is a well known wind pollinated genus, mainly distributed in temperate areas of mid to high latitudes of the northern hemisphere, colonizing in arid and semiarid environments landscape and has only few representatives in southern hemisphere (McArthur and Plummer, 1978; Valles and McArthur, 2001). Many species of the genus have a high economic value as medicines, food, forage, ornamentals and soil stabilizers in disturb habitats; some taxa are toxic or allergenic and some others are invasive weeds which can adversely affect harvests (Pareto, 1985; Tan et al., 1998). Most species in the genus are perennial; only approximately 10 species are annuals or biannual (Valles et al., 2003). *Artemisia* is also considered as indicator of steppe climate (Erdtman, 1952) and moderate precipitation (El-Moslimany, 1990).

After various taxonomic rearrangements (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 divided into five large groups which have been considered at sectional or subgeneric level; *Absinthium* (Mill.) DC., *Artemisia* (=*Abrotanum* Duhamel), *Dracunculus* Besser, *Seriphidium* Besser and *Tridantatae* (Rydb.) McArthur which is only restricted to North America. Ling (1991a, 1995b) separated *Seriphidium* from *Artemisia* as a new genus. Bremer (1994) accepted this separation but Torrell et al. (1999) and Watson et al. (2002) again united *Seriphidium* with *Artemisia*. But still a clear picture of natural classification within *Artemisia* has not been portrayed.

Pollen morphology of *Artemisia* has been studied from the time of Wodehouse (1926). The Subsequent workers such as Monoszon (1948, 1950a, 1950b), Straka (1952), Stix (1960), Singh and 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 et al. (2005) reveled different aspects of *Artemisia* pollen morphology including the structure, size and shape of pollen, sculptural types of exine, aperture dimensions, etc. along with their systematic relevance.

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Pollen grains of this genus are more or less distinct and easily recognized and are characterized by short spines or lack of spines (Bremer and Humphries, 1993). They are small in size but large in amount (Jiang et al., 2005). Martin et al. (2001; 2003) carried out a palynological study on the tribe Anthemideae including *Artemisia*, its allies and segregate genera and determined that ornamentation with short spinules is good taxonomic marker for *Artemisia* and its allies. It seems that the pollen morphology of *Artemisia* has remained unchanged throughout its documented history, as indicated by morphological comparisons between fossils and modern pollen grains (Wang, 2004). Wang (2004) also demonstrate 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 past.

The present work is the first detailed pollen grain study of the genus *Artemisia* in the South Asian region representing *Artemisia* species form Pakistan. In this research we mainly focused on the systematic potentials of micromorphological traits of pollen for phylogenetic allegations within the genus.

MATERIALS AND METHODS

Plant material

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. These pollen samples represent all the sections of *Artemisia* except *Tridentateae* which is restricted to North America and is not a part of phytogeographic assemblage that we focused in this study. Origin and detail of studied taxa is given in Table 1.

Methods

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; 2007) and Bibi et al. (2008). For SEM pollen grains were acetolysed and directly transferred to the aluminum stub and coated with gold in a sputtering chamber (SPI-Module Sputter Coater). Jeol-JSM 5910 scanning electron microscope was operated at 5 kV, at Centralized Resource Laboratory, Department of Physics, University of Peshawar, Pakistan. For LM the pollen grains were mounted on glass slide in saffranin stained glycerin jelly and sealed with transparent nail polish. 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 measurements from each specimen. Observations for polar diameter (P), equatorial diameter (E) and P/E ratio were taken such a way that for each parameter, the arithmetic mean has been calculated according to Erdtman (1952) and Reitsma (1970).

Phylogenetic analysis

On the bases of LM and SEM observations, nine micromorphological characters of pollen grains were selected as a character states for phylogenetic analysis of *Artemisia* (Table 2). To unveil the infra-specific variability of the pollens the continuous character states are considered in order of their degenerative appearance (Table 2, characters 6 - 9). The plesiomorphic or apomorphic state of each character was determined using the criteria established by Martin et al. (2001 and 2003) and further explained by Jiang et al. (2005). In this study an imaginary outgroup was used for comparison that contained all the ancestral characters. An original data matrix was produced using the outgroup comparison method (Watrous and Wheeler, 1981) (Table 3). For phylogenetic analysis the original data matrix was transformed into binary data matrix by FACTOR program of PHYLIP computer software version 3.67 (Felsenstein, 2007). The most parsimonious trees (MPTs) based on the binary matrix were constructed with MIX program of PHYLIP using Wagner parsimony method (Farris, 1970). A strict consensus phylogenetic tree of the MPTs was generated using the CONSENSE program of PHYLIP (Sokal and Rohlf, 1981). Finally, using DRAWTREE and DRAWGRAM programs of PHYLIP strict consensus phylogenetic tree of *Artemisia* were generated.

RESULTS AND DISCUSSION

From LM and SEM (Figures 1 and 2) observations it is obvious that the shape of pollen grain is homogeneous throughout in the genus and supports its monophyly. 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 et al. (2005). The exine of the pollen has a noticeable double layered structure of inner and outer layers with aggregated columella.

From 79 MPTs, a strict consensus phylogenetic tree was obtained (Figure 3). In this phylogenetic tree, *Artemisia* and *Seriphidium* are shown to be a monophyletic group as indicated in molecular studies of Kornkven et al. (1998), Torrell et al. (1999) and Watson et al. (2002). Figure 3 also compare evolutionary pattern of *Artemisia* Pollens with traditional classification which was based only on floral characters and has many objections as for example section *Artemisia* only differ from section *Absinthium* by a single character, that is, receptacle naked (*Artemisia*) or receptacle cover with long hairs (*Absinthium*) (Kaul and Bakshi, 1984).

The tendency of pollen morphological evolution is to develop more and more degenerative features. 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, that is, 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

Table 1. List of taxa studied for palynology and their herbarium vouchers. ISL: Herbarium, Quaid-i-Azam University, Islamabad. PUP, Herbarium, University of Peshawar, Peshawar.

Taxon	Collection data	Herbarium Voucher
Section *Artemisia* Tournefort		
A. amygdalina Decne.	Mansehra: Naran to Lake Saif-ul-Malook track. T. Malik, 1972.	ISL, 32315
A. biennis Willd.	Rawalpindi: Murree Hills, PLT, Ayubia National Park. M. Q. Hayat, 2007.	PUP, PH005 (ART005)
A. dubia Wall. ex Besser	Rawalpindi: Murree Hills, PLT, Ayubia National Park. M. Q. Hayat, 2007.	PUP, PH002 (ART002)
A. gmelinii Web. ex Stechm	Gilgit: Hispar. M. Zaffar, 2006.	ISL, 23441
A. moorcroftiana Wall. ex DC.	Azad Jummu and Kashmir: Muzafrabad. T. Malik, 1972.	ISL, 26550
A. roxburghiana Wall. ex Besser	Rawalpindi: Murree Hills, PLT, Ayubia National Park. M. Q. Hayat, 2007.	PUP, PH001 (ART001)
A. rutifolia Spreng.	Gilgit: Nattar valley. A. Rashid, 1988.	PUP, 244 (1105)
A. santolinifolia Turcz. ex Krasch.	Gilgit: Nattar valley. A. Rashid, 1986.	PUP, 239 (1108)
A. tournefortiana Reichenbach	Rawalpindi: Murree Hills, PLT, Ayubia National Park. M. Q. Hayat, 2007.	ISL, 21921
A. vestita Wall. ex DC.	Jahlum: Soon vally, Sakasar. M. Farooq, 2005.	ISL, 20093
A. vulgaris L.	Azad Jummu & Kashmir: Pearl valley, Mutyal Mara. M. Q. Hayat, 2008.	PUP, PH006 (ART006)
Section *Absinthum* (Mill.) DC		
A. absinthium L.	Gilgit: Nattar valley. M. Q. Hayat, 2007.	PUP, PH004 (ART004)
A. siversiana Ehrh.	Gilgit: Nattar valley. A. Rashid, 1986.	PUP, 222 (1057)
A. tangutica Pampanini	Gilgit: Hunza vally. M. Q. Hayat, 2007.	ISL, 32144
Section *Seriphidium* (Besser) Besser		
A. herba-alba Asso.	Quetta: Hanna Lake. M. A. Khan, 2007	ISL, 28615
S. brevifolium Wall. ex DC.	Mansehra: Ujtar, Naran to Lalusar lake track. M. Q. Hayat, 2007.	PUP, PH007 (ART007)
S. kurramense (Qaz.) YR Ling	Kurram Agency: Burki. N. A. Qazilbash, 1937.	PUP, 22419
S. turanicum (Krasch.) Poljakov	Gilgit: Nattar vally. M. Q. Hayat, 2008.	PUP, PH009 (ART009)
Section *Dracunculus* Besser		
A. desertorum Spreng.	Gilgit: Mahthantir Gah. T. Malik, 1972.	ISL, 25115
A. japonica Thunb.	Rawalpindi: Murree Hills, PLT, Ayubia National Park. M. Q. Hayat, 2007.	PUP, PH008 (ART008)
A. scoparia Waldst. et Kit.	Islamabad: Quaid-i-Azam university campus. M. Q. Hayat, 2008.	ISL, 32313
A. stricta Edgew.	Kashmir: Muzafrabad. T. Malik, 1972.	ISL, 25650

low latitude and low evaluation moist regions during the glacial epoch (Wang, 2004; Jiang et al., 2005).

There were four sections in the classical classifications within the genus *Artemisia* (Hooker, 1881; McArthur et al., 1981; Ling, 1982, 1991a, 1991b, 1995a, 1995b; Bremer, 1994). In this study, we supports the reunion of the *Artemisia* and *Seriphidium* based on the micromorphological characteristics of pollen grains as pointed out in molecular studies of Kornkven et al. (1998), Torrell et al. (1999) and Watson et al. (2002). Our study also reveals that member of different sections of *Artemisia* formed sisters groups with other sections and raising the idea of polyphyly of these sections (Figure 3). It also seems that more taxa with sufficient number of representatives from each classical section are needed to be sampled

Table 2. Characters and character states of pollen grains for the phylogenetic analysis of *Artemisia*. The number in brackets represents the codes of character states. The code of plesiomorphic character state is always 0.

Characters		Character states
1.	Pollen type	*Anthemis* (0), *Artemisia* (1)
2.	Pollen shape	Globular (0), Oblate (1)
3.	Spinules arrangement	Dense (0), Loose (1)
4.	Exine sculpture	Granular (0), Sinuolate (1)
5	Spinules base	Stretching and outward extending (0) Normal (1)
6	Polar length	>26 µm (0), >23 - 26 µm (1), >22 – 23 µm (2), >21 - 22 µm (3), >20 - 21 µm (4), >19 - 20 µm (5), >18 - 19 µm (6), >17 – 18 µm (7), 16 – 17 µm (8)
7	Equatorial width	>21 µm (0), >20 – 21 µm (1), >19 – 20 µm (2), >18 - 19 µm (3), >17 – 18 µm (4), >16 – 17 µm (5), >15 – 16 µm (6) 14 – 15 µm (7)
8	Exine thickness	>3 µm (0), 2 – 3 µm (1)
9	Colpus width	>14 µm (0), >13 – 14 µm (1), >12 – 13 µm (2), >11 – 12 µm (3), >10 – 11 µm (4), 9 – 10 µm (5)

Table 3. Character state matrix used in phylogenetic analysis of *Artemisia*. Characters and character states are described in Table 2.

Taxa	Abbreviation	1	2	3	4	5	6	7	8	9
A. amygdalina	AMY	1	1	1	1	1	5	1	1	0
A. biennis	BIE	1	1	1	1	1	4	3	1	3
A. dubia	DUB	1	1	0	0	1	2	2	0	1
A. gmelinii	GME	1	1	1	1	1	3	5	0	3
A. moorcroftiana	MOO	1	0	0	4	0	0	0	0	0
A. roxburghiana	ROX	1	0	1	1	1	3	1	1	1
A. rutifolia	RUT	1	0	1	0	0	4	3	0	2
A. santolinifolia	SAN	1	0	1	1	0	3	4	1	2
A. tournefortiana	TUR	1	0	1	1	1	6	3	1	2
A. vestita	VES	1	0	1	0	0	5	3	1	3
A. vulgaris	VUL	1	1	0	1	0	3	4	1	3
A. absinthium	ABS	1	0	1	1	1	4	5	0	2
A. siversiana	SIV	1	0	0	1	0	7	5	1	3
A. tangutica	TAN	1	1	0	0	1	4	0	1	1
A. herba-alba	HER	1	0	1	0	1	5	3	1	3
S. brevifolium	BRE	1	1	1	1	1	2	4	1	1
S. turanicum	TRN	1	1	1	1	1	3	3	1	3
S. kurramense	KUR	1	1	1	1	1	1	2	1	2
A. desertorum	DES	1	1	1	0	1	6	6	1	4
A. japonica	JAP	1	0	1	0	1	6	3	0	1
A. scoparia	SCO	1	1	1	0	1	8	4	1	4
A. stricta	STR	1	1	1	1	1	8	7	1	5
Outgroup	OUT	0	0	0	0	0	0	0	0	0

and studied further especially using molecular approaches to reach an inclusive conclusion.

Conclusions

In the present study, it is evident that micromorphological features of pollen of *Artemisia* are phylogenetically potential traits for the genus and have great value to contribute in the debate of evolution and systematics of *Artemisia*. Genomic and other investigations such as phytochemistry, karyology, phytogeography are required to ascertain the subgeneric natural classification system of the genus *Artemisia*.

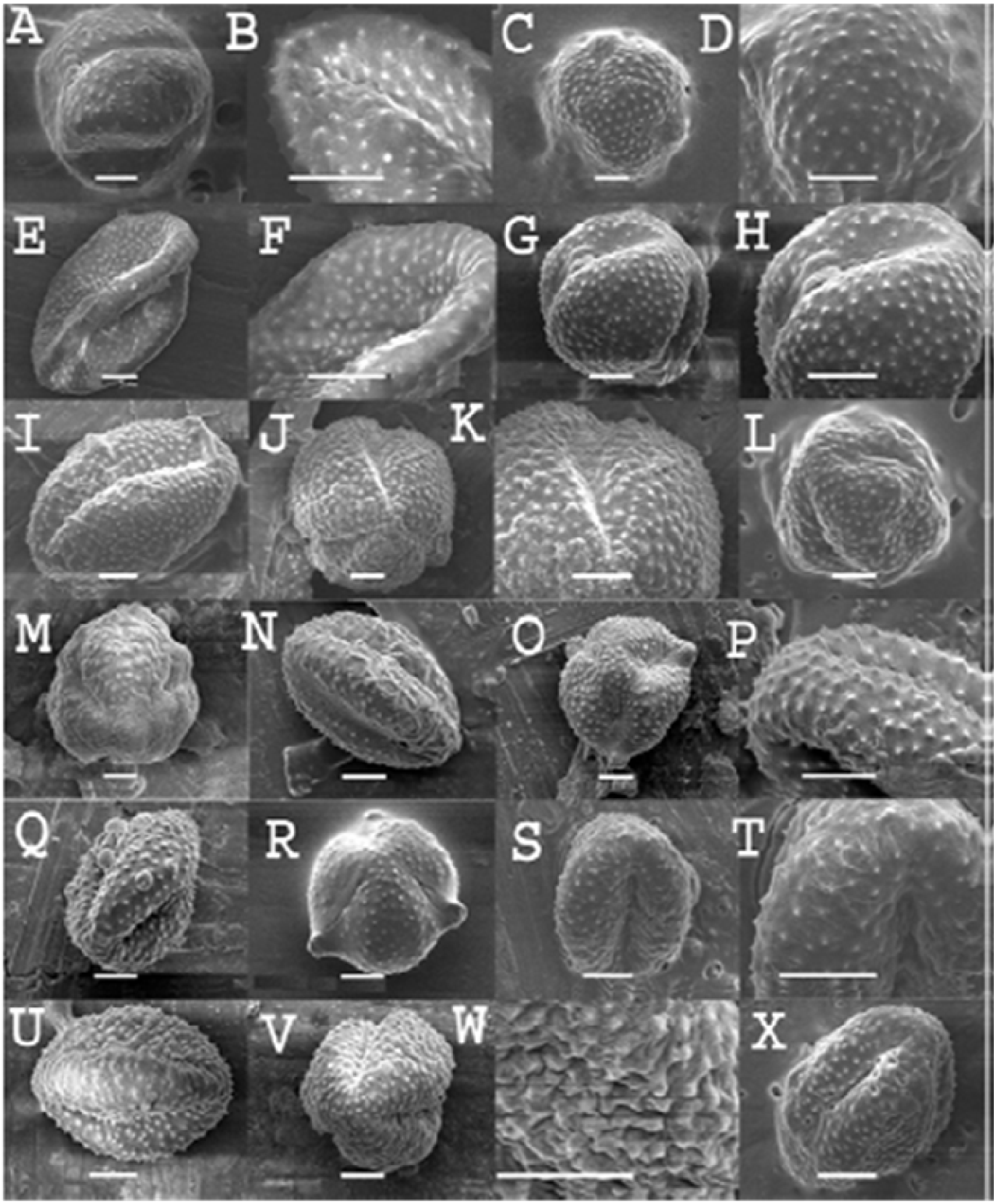

Figure 1. Scanning electron micrographs of the taxa studied: *A. amygdalina* (A, B), *A. biennis* (C, D), *A. dubia* (E, F), *A. gmelinii* (G, H), *A. moorcroftiana* (I, J, K), *A. roxburghiana* (L), *A. rutifolia* (M), *A. santolinifolia* (N, O, P), *A. tournefortiana* (Q, R), *A. vestita* (S, T), *A. vulgaris* (U, V, W) and *A. absinthium* (X) . Scale bar = 5 µm.

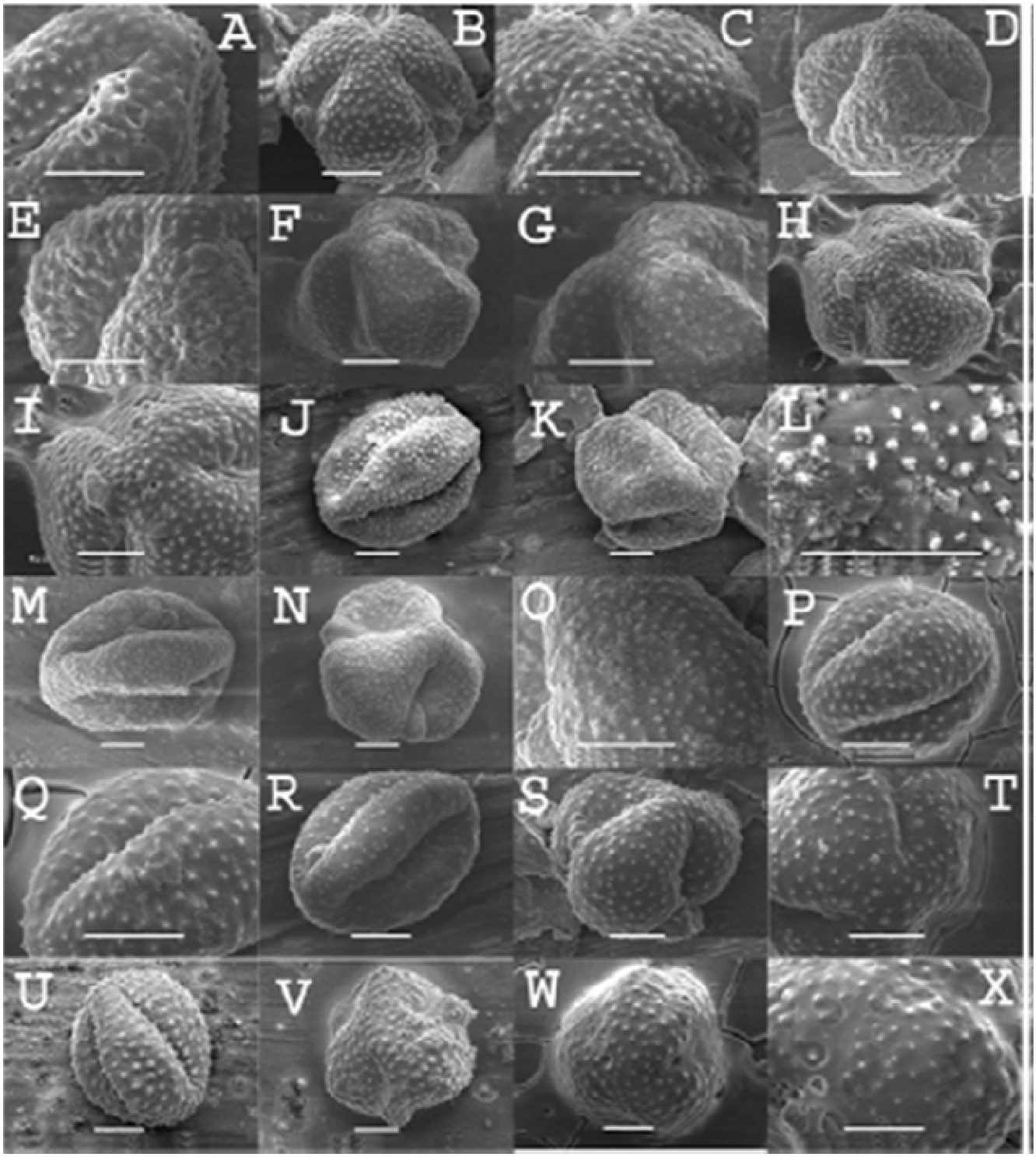

Figure 2. Scanning electron micrographs of the taxa studied: *A. absinthium* (A), *A. siversiana* (B, C), *A. tangutica* (D, E), *A. herba-alba* (F, G), *S. brevifolium* (H, I), *S. turanicum* (J, K, L), *S. kurramense* (M, N, O), *A. desertorum* (P, Q), *A. japonica* (R, S, T), *A. scoparia* (U, V) and *A. stricta* (W, X) . Scale bar = 5 µm.

ACKNOWLEDGEMENTS

We are thankful to Dr. Riaz, Department of Physics, for his co-operation. We are also thankful to Dr. M. Tahir Shah, NCE in Geology, University of Peshawar, Pakistan for his facilitation. We also acknowledge HEC Pakistan

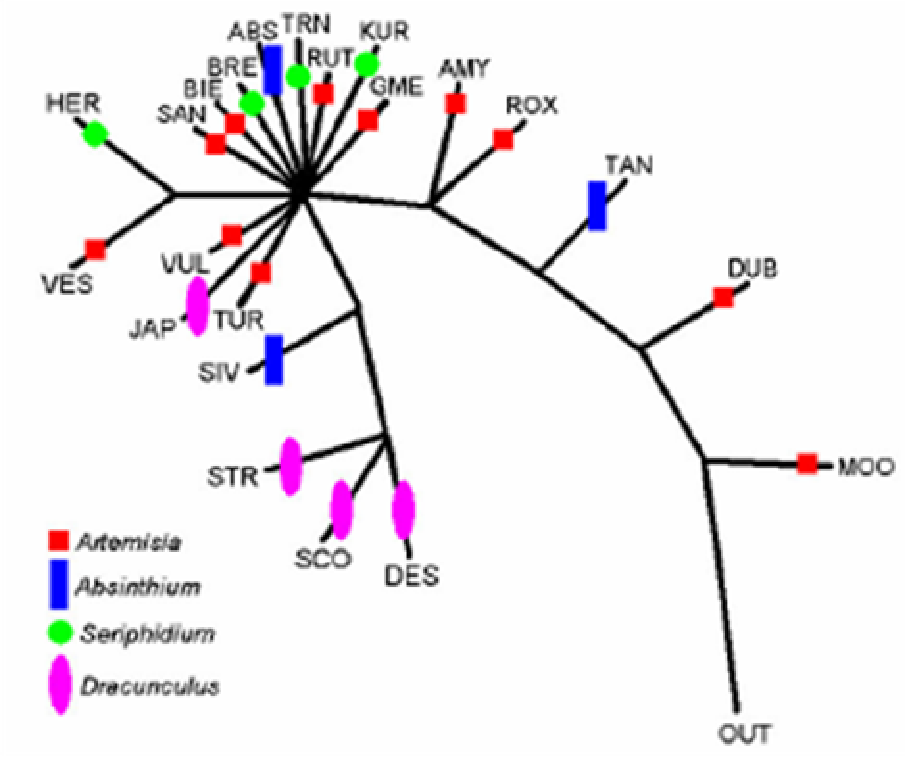

Figure 3. The strict consensus phylogenetic tree of *Artemisia* based on the micromorphological characters of pollen grains. Latter represents the abbreviations of taxa (see Table 3). The tree was rooted with imaginary outgroup. Coloured geometrical shapes indicate the classical sectional division of the genus based on floral morphology.

for their financial assistance.

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