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RESEARCH ARTICLE

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Application and implication of scanning electron microscopy for evaluation of palyno-morphological features of Vitaceae from Pakistan

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Abstract

The present study aimed to investigate the palyno-morphological features of species of family Vitaceae from Pakistan. A total of nine species, belonging to four genera were collected, pressed, identified, and then analyzed microscopically. Both quantitative and qualitative characters of the pollen grains were recorded including polar and equatorial diameter, P/E ratio, number of colpi and pores, exine thickness and shapes of the pollen in both polar and equatorial view, and exine sculpturing using Leica microscope fitted with camera Meiji Infinity 1 and then analyzed statistically using software IBM SPSS Statistics 20. The results of the present study demonstrated the variations in polar and equatorial diameter, exine thickness, P/E ratio, pollen shape, and exine sculpturing of the studied species and highlighted the significance of pollen morphology as an identification tool. The present study may contribute to better understand the classification at genus level, which will support the future phylogenetic characterization of the family.

KEYWORDS

morphology, pollen grains, scanning electron microscopy, Vitaceae

1 | INTRODUCTION

The grape family, Vitaceae, is comprised of around 700 species worldwide, belonging to 15 genera, mostly distributed in tropical, subtropical, and temperate regions. The family is characterized by simple, alternate, lobed or unlobed, or pinnately compound leaves with opposite tendrils, panicles to corymbs inflorescence, and unarmed stems (Lombardi, 2007; Najmaddin, 2014a). Most of the members are lianas or woody creepers, some members are herbaceous, few are shrubs and rarely trees, treated under order Vitales with further subdivision into 4-merous and 5-merous groups. Commercially, it is a well-known family among the fruit-producing plants, especially for the economic importance of the grapes primarily characterized as a food source and also for wine and resin production (Karkamkar, Patil, & Misra, 2010).

In Pakistan, family is represented by 6 genera and 12 species containing copious watery juices, unisexual or bisexual, small and actinomorphic flowers, four to five united sepals, two carpels, syncarpous, fruit is berry, one to four seeds per berry. The chief genera of the family Vitaceae are *Vitis* (grape), *Parthenocissus* (Virginia creeper), *Ampelopsis* (pepper vine), *Ampelocissus*, and *Cissus* (Perveen & Qaiser, 2008).

Vitaceae species are distinguished largely on the basis of their floral features with limited knowledge regarding botanical and commercial purpose of the family. The only economically important species is *Vitis vinifera* that is being utilized for the wine production. Some species of *Parthenocissus*, known for their ornamental use because of their attractive leaf color in autumn are *Parthenocissus quinquefolia* and *Parthenocissus tricuspidata*. These are fast-growing deciduous vines, commonly known as Virginia creeper, climbing by adhesive disks and tendrils (Najmaddin, 2014a).

The pollen morphology of the species of genus *Cissus* revealed that the majority of the members have medium-sized tricolporate pollen with varied sexine ornamentation confirming the heterogeneity of pollen grain, which helped greatly in distinguishing the species and also the delimitation of the taxa. The palyno-morphology may contribute to better understand the classification of the genus, which will support the future phylogenetic characterization of the family (Cartaxo-Pinto, Mendonça, Lopes, & Gonçalves-Esteves, 2017).

The revision of field of palynology to reexamine its role in angiosperm systematics and phylogenetic studies confirmed the significance of principal pollen characters which are phylogenetically applicable at higher taxonomic ranks are colpus type, pollen shape, grain size, pollen wall sculpturing, pollen dispersal unit, polarity, symmetry, and many other morphological features. These palyno-morphological features and concepts are in correspondence with the higher taxonomic ranks of flowering plants in Cronquist and Takhtajan systems of classification and aids in the phylogenetic analysis. Pollen morphology correlates with the ranks of these two systems of classifications with few exceptions (Walker & Doyle, 1975).

Majority of the species are very rare in Pakistan and distributed distantly across the country. Common folk is ignorant of the distribution, appearance, and nutritional value of Vitaceae species except grapes (*Vitis vinifera*) which is cultivated. It has been noticed that plants belonging to the family are known with similar local names or different names for the same species thus creating misidentification and confusion regarding species for the researchers. There is a need for systematic exploration of Vitaceae taxa along with morphological, anatomical, and palynological features for correct identification and delimitation. The present study aimed to examine the detailed pollen morphology including shape, size, exine ornamentation of Vitaceae species from Pakistan, using light microscopy (LM) and scanning electron microscopy (SEM) for the correct identification of the species, which may lead to the better classification of the family and advancement in phylogenetic tree.

2 | MATERIALS AND METHODS

2.1 | Plant collection

Plant material used in this study comprised of nine species belonging to four genera, including *Vitis jacquemontii* R. Parker, *Vitis vinifera* L., *Vitis flexuosa* Thunb., *Parthenocissus quinquefolia* (L.) Planch., *Parthenocissus semicordata* (Wall.) Planch., *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch., *Ampelopsis vitifolia* subsp. *hazaraganjiensis* Nazim & Qaiser, *Cissus trifoliata* (L.) L., and *Cissus quadrangularis* L. The plant species were collected from various localities of Pakistan during the field trips conducted from April to September 2017. During the field trips, plants were photographed and carefully collected (Figures 1 and 2). Mature flowers were selected for pollen morphological studies. Plant species were identified using the Flora of West Pakistan (Nasir, Ali, & Stewart, 1972) and species names were identified from the Kew botanical garden correct scientific name service The Plant List (www.theplantlist.org). After confirmation, the plants were properly pressed and dried for further experimentation at Plant Systematics and Biodiversity Laboratory, Quaid-i-Azam University Islamabad, Pakistan. Authentic botanical names, voucher specimen number, English name, domestication status, collection site, worldwide and distribution in Pakistan are presented in Table 1.

2.2 | Pollen micromorphology using LM

The pollen grains were prepared for LM according to the modified methodology of Bahadur et al. (2019). From the mature flowers, the pollen grains were separated and kept on the slides. The pollen grains were treated with 99.9% pure acetic acid and then crushed with a glass rod so that pollen can come out from the anthers of the flowers. The debris was removed, and pollen was treated with glycerine jelly, covered with coverslips, and then studied with the help of light microscope Meiji Techno MT4300H. Both quantitative and qualitative characters were studied and presented in tabular form including pollen shape, size, ornamentation, polarity, polar diameter, equatorial diameter, exine thickness, colpi number, colpi length, colpi width, and P/E ratio (polar to equatorial ratio). Freshly prepared slides were photographed with the help of Leica microscope fitted with camera Meiji Infinity 1 (Table 2).

2.3 | Pollen micromorphology using SEM

SEM of pollen was performed by following the methodology of Butt et al. (2018), with slight modification. The mature anthers were dissected and placed in the middle of a clean glass slide and treated with 1–2 drops of acetic acid for 1 min. The anthers were crushed to release pollen and then transferred to pre-marked specimen stub where they were allowed to air dry. After some time, they were coated with gold via SPI-MODEL™ sputter-coater, then stubs were positioned in Jeol Vacuum evaporator. It took about 15 min to form the vacuum and then observations were made using 30 kV SEM (J.S. M-5910, JEOL, Japan).

2.4 | Statistical analysis

To find average, five consecutive values were noted. Mean value and standard for each feature were calculated by using statistical software

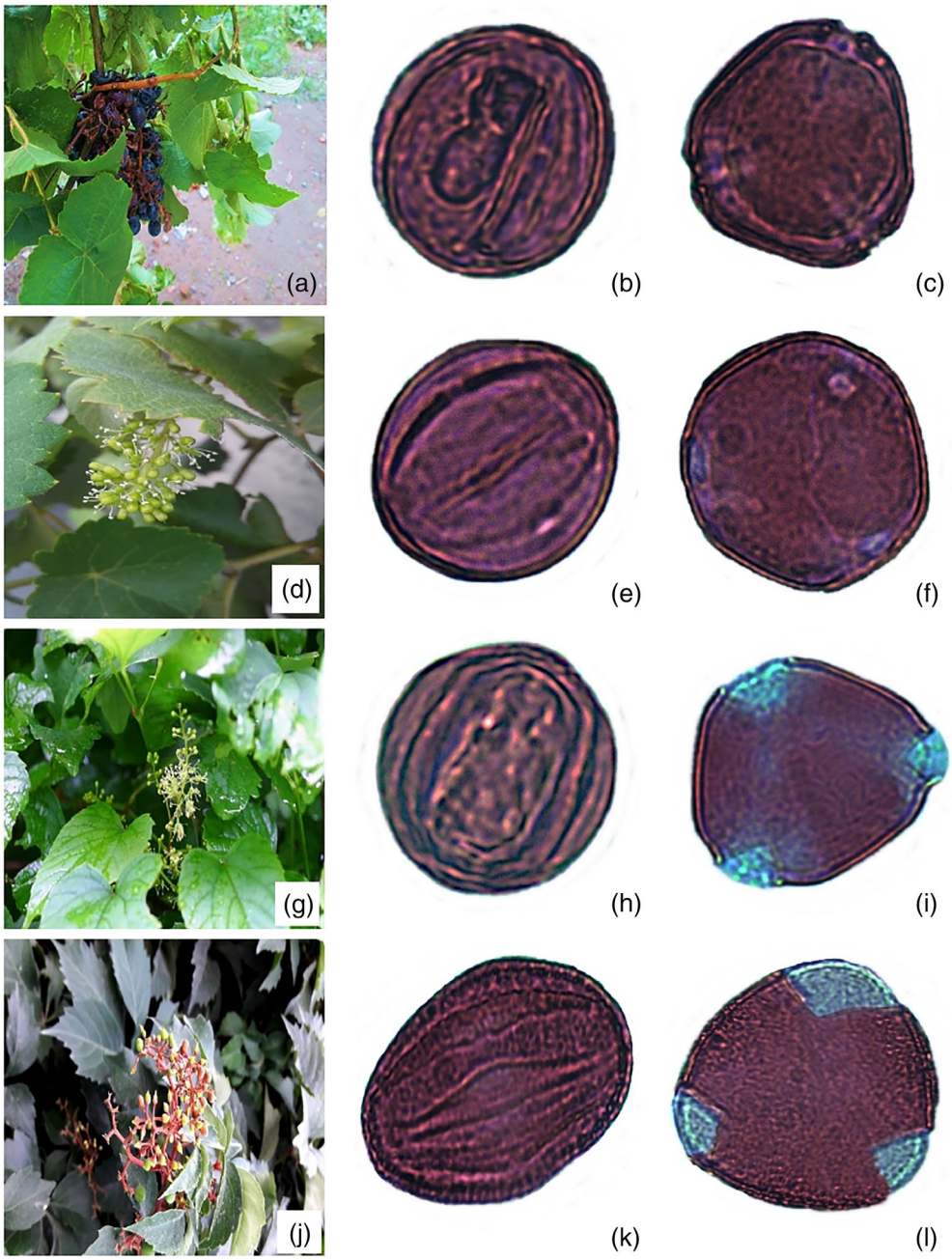

FIGURE 1 Vitis jacquemontii (a) Field photograph, light micrographs (LM) of pollen grains—(b) equatorial view, (c) polar view; *Vitis vinifera*. (d) Field photograph, light micrographs (LM) of pollen grains—(e) equatorial view, (f) polar view; Vitis flexuosa. (g) Field photograph, light micrographs (LM) of pollen grains—(h) equatorial view, (i) polar view; *Parthenocissus quinquefolia*. (j) Field photograph, light micrographs (LM) of pollen grains—(k) equatorial view, (l) polar view [Color figure can be viewed at wileyonlinelibrary.com]

IBM SPSS Statistics 20. Values are presented as mean (minimum-maximum) ± standard error in Table 3.

3 | RESULTS

3.1 | *Vitis jacquemontii* R. Parker

Grains are monad, small sized, isopolar, trigonal in polar view, oblate-spheroidal in shape, and tricolporate with rugulate-reticulate exine sculpturing. Average polar diameter is 14.52 ± 0.63 μm, equatorial diameter is 15.69 ± 0.96 μm, P/E ratio is 0.93 μm, exine thickness is 1.56 ± 0.11 μm, colpi length is 3.81 ± 3.81 μm, and width is 2.55 ± 0.53 μm (Figures 1b,c and 3a–c).

3.2 | *Vitis vinifera* L.

Pollen is monad, small sized, isopolar, circular to trigonal in polar view, prolate-spheroidal in shape, and tricolporate with reticulate sculpturing. Average polar diameter is 22.29 ± 1.11 μm, equatorial diameter is 20.13 ± 1.02 μm, P/E ratio is 1.11 μm, exine thickness is 1.47 ± 0.09 μm, colpi length is 5.28 ± 1.01 μm, and width is 6.60 ± 1.70 μm (Figures 1e,f and 3d–f).

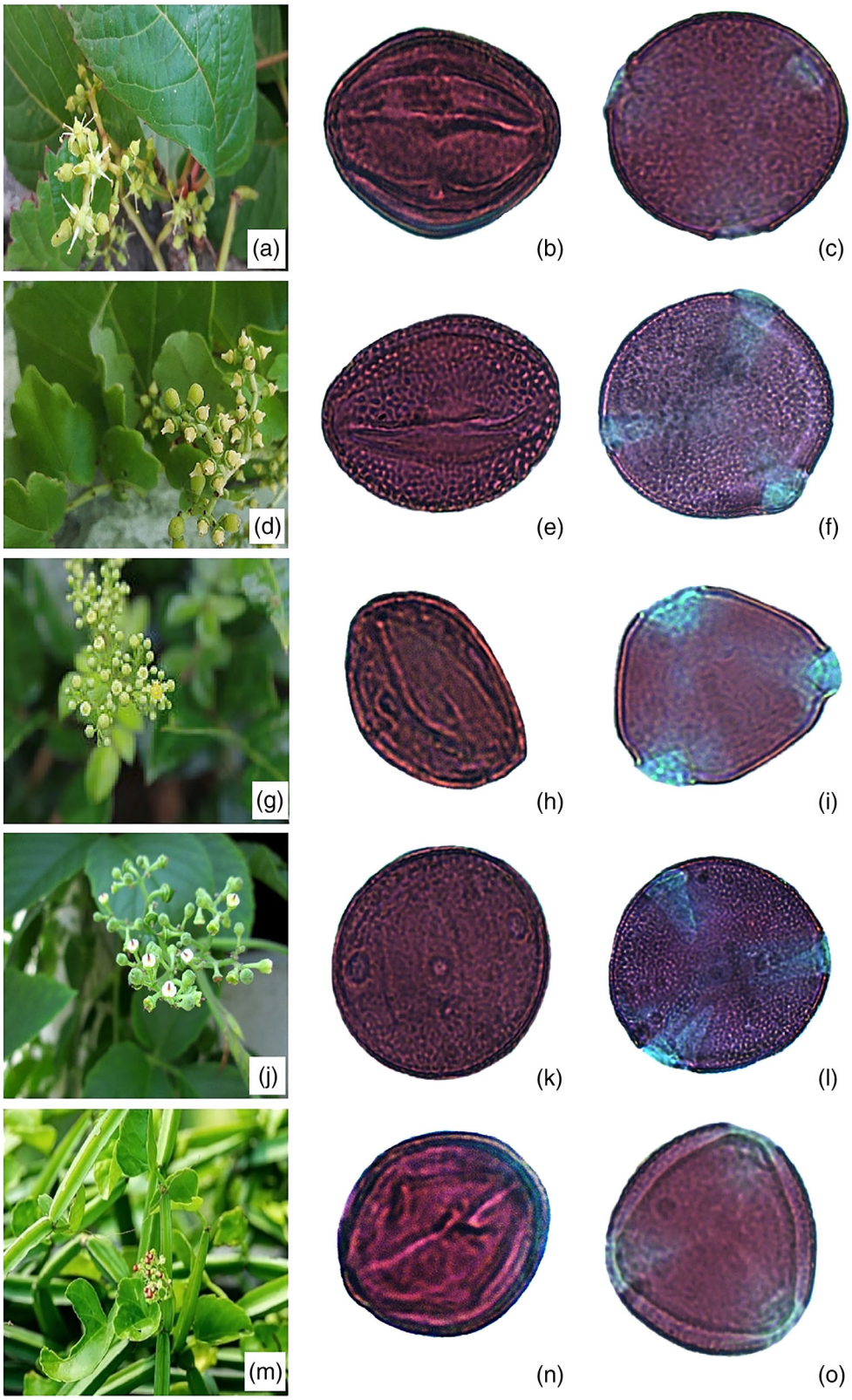

FIGURE 2 Parthenocissus semicordata (a) Field photograph, light micrographs (LM) of pollen grains—(b) equatorial view, (c) polar view; *Parthenocissus tricuspidata* (d) Field photograph, light micrographs (LM) of pollen grains—(e) equatorial view, (f) polar view; Ampelopsis vitifolia subsp. hazaraganjiensis (g) Field photograph, light micrographs (LM) of pollen grains—(h) equatorial view, (i) polar view; *Cissus trifoliata* (j) Field photograph, light micrographs (LM) of pollen grains—(k) equatorial view, (l) polar view; *Cissus quadrangularis* L. (m) Field photograph, light micrographs (LM) of pollen grains—(n) equatorial view, (o) polar view [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | *Vitis flexuosa* Thunb.

Pollen grain is monad, small sized, isopolar, trigonal in polar view, prolate-spheroidal in shape, and tricolpate with foveolate sculpturing. Average polar diameter is 18.03 ± 0.34 μm, equatorial diameter is 17.85 ± 0.84 μm, P/E ratio is 1.01 μm, exine thickness is 1.80 ± 0.23 μm, colpi length is 6.12 ± 1.40 μm, and width is 3.45 ± 0.97 μm (Figures 1h,i and 3g–i).

TABLE 1 List of plants collected and collection site

Plant name	Synonyms	Local name	English name	Collection site	Coordinates		Distribution in Pakistan	Worldwide distribution
					Latitude	Longitude		
Vitis jacquemontii R. Parker	*Vitis lanata* Roxb.	Gidar dakh	Jackal grapes	Khaki, Mansehra	34.40203°N	73.160398 °E	Balakot, Mansehra, Shinkiari, Baffa, Khaki, Battal, Torghar, Abbottabad, Swat, Bahrain, Marghuzar	Sub-Himalayan tracts of Pakistan, India, and China
Vitis vinifera L.	*Cissus vinifera* (L.) Kuntze	Angur	Grapes	Baidra, Mansehra	34.332879°N	73.126616 °E	Pishin, Quetta, Chitral, Swat, Abbottabad, Mansehra, Gilgit Baltistan, Astor, Skardu, Kashmir, Kurram	Afghanistan, Austria, Bulgaria, China, Czechoslovakia, France, Germany, Greece, Hungary, Iran, Italy
Vitis flexuosa thumb.	*Vitis parvifolia* Roxb.	Jungli dakh	Creeping grape	Matta, Swat	34.93712°N	72.414796 °E	Poonch, Bagh, Trarkhel, Swat, Bahrain, Matta, Mansehra, Kohistan, Battagram, Pakhal, Alai	Bangladesh, China, Japan, India Indonesia, Malaysia, Korea, Nepal, Philippines, Taiwan, Thailand, Vietnam
Parthenocissus quinquefolia (L.) Planch.	*Ampelocissus hederacea* DC.	Panjpatti bail	Virginia creeper	Kaghan Bazaar, Mansehra	34.780963°N	73.5219 °E	Hazara Dist.: Jinnah Garden, Abbottabad, Mansehra, Kaghan, Swat	America, Austria, Algeria, China, Germany, Greece, Korea, Italy, Switzerland
Parthenocissus semicordata (Wall.) Planch.	*Vitis himalayana* (Royle) Brandis	Bilri	Himalayan woodbine	Ayubia National Park, Ayubia, Abbottabad	34.06192°N	73.402264 °E	Swat, Kaghan valley, Nathia gali, Jhika gali, Murree, Dunga gali, Bara gali, Kashmir	Bangladesh, China, Malaysia, Malaya, Indonesia, Myanmar, Nepal, Thailand, Vietnam
Parthenocissus tricuspidata (Siebold & Zucc.) Planch.	*Ampelopsis tricuspidata* Siebold & Zucc.	Chinar bail	Boston ivy	Jinnah Abad, Abbottabad	34.193647°N	73.237474 °E	Abbottabad, Balakot, Qalandarabad, Mansehra, Jabbori, Islamabad	Belgium, China, Columbia, Japan, Korea, Russia, Taiwan, Vietnam
Ampelopsis vitifolia subsp. *hazaraganjiensis* Nazim & Qaiser	–	–	Pepper vine	Hazarganji National Park, Quetta	30.313144°N	66.815881 °E	Hazarganji, Kach, Saru Range, Shaldara, Quetta	Endemic to Pakistan
Cissus trifoliata (L.) L.	*Cissus carnosa* Lam.	Sorrel vine	–	Saidpur, Islamabad	33.742894°N	73068366 °E	Abbottabad, Havelian, Murree, Saidpur Islamabad, Salt Range, Taxila	America, Bangladesh, China, India, Jamaica, Myanmar, Nepal
Cissus quadrangularis L.	*Cissus bifida* Schumach & Thonn.	Harjora	Veldt grape	Hyderabad, Karachi	25.443669°N	68.332452 °E	Karachi, Hyderabad, Tando Jam	Bangladesh, India, Kenya, Nigeria, Oman, Philippines, Somalia, Sudan, Turkey

TABLE 2 Qualitative palyno-morphological characters of studied species of Vitaceae

S. No.	Taxa	Polar view	Dispersal unit	Pollen size	Pollen shape	Colpi P/A	Pore P/A	Number of Colpi	Exine sculpturing	Polarity
1	*Vitis jacquemontii* R. Parker	Trigonal	Monad	Small	Oblate-spheroidal	P	P	Tricolporate	Rugulate-reticulate	Isopolar
2	*Vitis vinifera* L.	Circular-trigonal	Monad	Small	Prolate-spheroidal	P	P	Tricolporate	Reticulate	Isopolar
3	*Vitis flexuosa* Thunb.	Trigonal	Monad	Small	Prolate-spheroidal	P	A	Tricolpate	Foveolate	Isopolar
4	*Parthenocissus quinquefolia* (L.) Planch.	Trigonal	Monad	Medium	Prolate-spheroidal	P	P	Tricolporate	Reticulate	Isopolar
5	*Parthenocissus semicordata* (Wall.) Planch.	Circular	Monad	Medium	Prolate-spheroidal	P	A	Tricolpate	Foveolate	Isopolar
6	*Parthenocissus tricuspidata* (Siebold & Zucc.) Planch.	Circular	Monad	Medium	Prolate-spheroidal	P	P	Tricolporate	Perforate	Isopolar
7	*Ampelopsis vitifolia* subsp. *hazaraganjiensis* Nazim & Qaiser	Trigonal	Monad	Medium	Subprolate	P	A	Tricolpate	Reticulate	Isopolar
8	Cissus trifoliata (L.) L.	Trigonal	Monad	Medium	Oblate-spheroidal	P	P	Tricolporate	Reticulate-perforate	Isopolar
9	*Cissus quadrangularis* L.	Circular	Monad	Small	Oblate	P	A	Tricolpate	Reticulate-perforate	Isopolar

Abbreviations: A, absent; P, present.

TABLE 3 Quantitative palyno-morphological characters of studied species of Vitaceae

		P/E ratio	Exine thickness	Polar diameter	Equatorial diameter	L of colpi	W of colpi
S. No.	Taxa	M (Min-Max) SE (μm)					
1	*Vitis jacquemontii* R. Parker	0.93	1.56 (1.35–1.95) ± 0.11	14.52 (12.15–15.60) ± 0.63	15.69 (12.30–18.00) ± 0.96	3.81 (1.65–7.95) ± 3.81	2.55 (1.50–4.50) ± 0.53
2	*Vitis vinifera* L.	1.11	1.47 (1.20–1.80) ± 0.09	22.29 (19.95–25.95) ± 1.11	20.13 (17.85–23.10) ± 1.02	5.28 (2.55–7.65) ± 1.01	6.60 (3.30–13.35) ± 1.70
3	*Vitis flexuosa* Thunb.	1.01	1.80 (1.35–2.55) ± 0.23	18.03 (16.80–18.60) ± 0.34	17.85 (15.30–19.65) ± 0.84	6.12 (1.65–10.20) ±1.40	3.45 (1.95–7.20) ± 0.97
4	*Parthenocissus quinquefolia* (L.) Planch.	1.09	1.05 (0.90–1.20) ± 0.11	30.51 (27.15–35.10) ± 2.89	27.75 (26.55–29.70) ± 1.36	2.09 (3.15–8.85) ± 2.09	9.48 (5.10–13.05) ± 2.91
5	*Parthenocissus semicordata* (Wall.) Planch.	1.07	1.50 (1.20–1.80) ± 0 .11	28.83 (25.50–33.15) ± 1.65	27.06 (25.65–30.30) ± 1.2	10.05 (4.05–17.55) ± 2.17	6.24 (2.85–13.20) ± 1.92
6	*Parthenocissus tricuspidata* (Siebold & Zucc.) Planch.	1.07	1.23 (0.90–1.50) ± 0.09	29.94 (28.20–32.20) ± 0.83	28.03 (25.80–31.80) ± 1.08	8.28 (2.55–19.65) ± 2.97	6.69 (2.55–10.8) ± 1.43
7	*Ampelopsis vitifolia* subsp. *hazaraganjiensis* Nazim & Qaiser	1.20	2.60 (2.20–2.90) ± 0.20	30.43 (28.30–32.50) ± 1.21	25.30 (25.20–25.50) ± 0.09	22.10 (20.30–23.10) ± 0.90	16.20 (12.70–20.10) ± 2.10
8	Cissus trifoliata (L.) L.	0.94	1.14 (0.90–1.65) ± 0.35	24.19 (22.5–27.15) ± 2.1	25.71 (23.10–29.70) ± 2.52	12.21 (10.05–15.15) ± 2.44	5.79 (3.00–8.55) ± 2.31
9	*Cissus quadrangularis* L.	0.67	2.01 (1.80–2.10) ± 0.10	11.70 (11.40–11.90) ± 0.15	17.30 (17.2–17.50) ± 0.09	12.30 (9.30–15.10) ± 1.80	7.20 (5.70–9.20) ± 1.03

Abbreviations: L, length; M, mean; Max, maximum; Min, minimum; P/E, polar to equatorial ratio; SE, standard error; μm, micrometer; W, width.

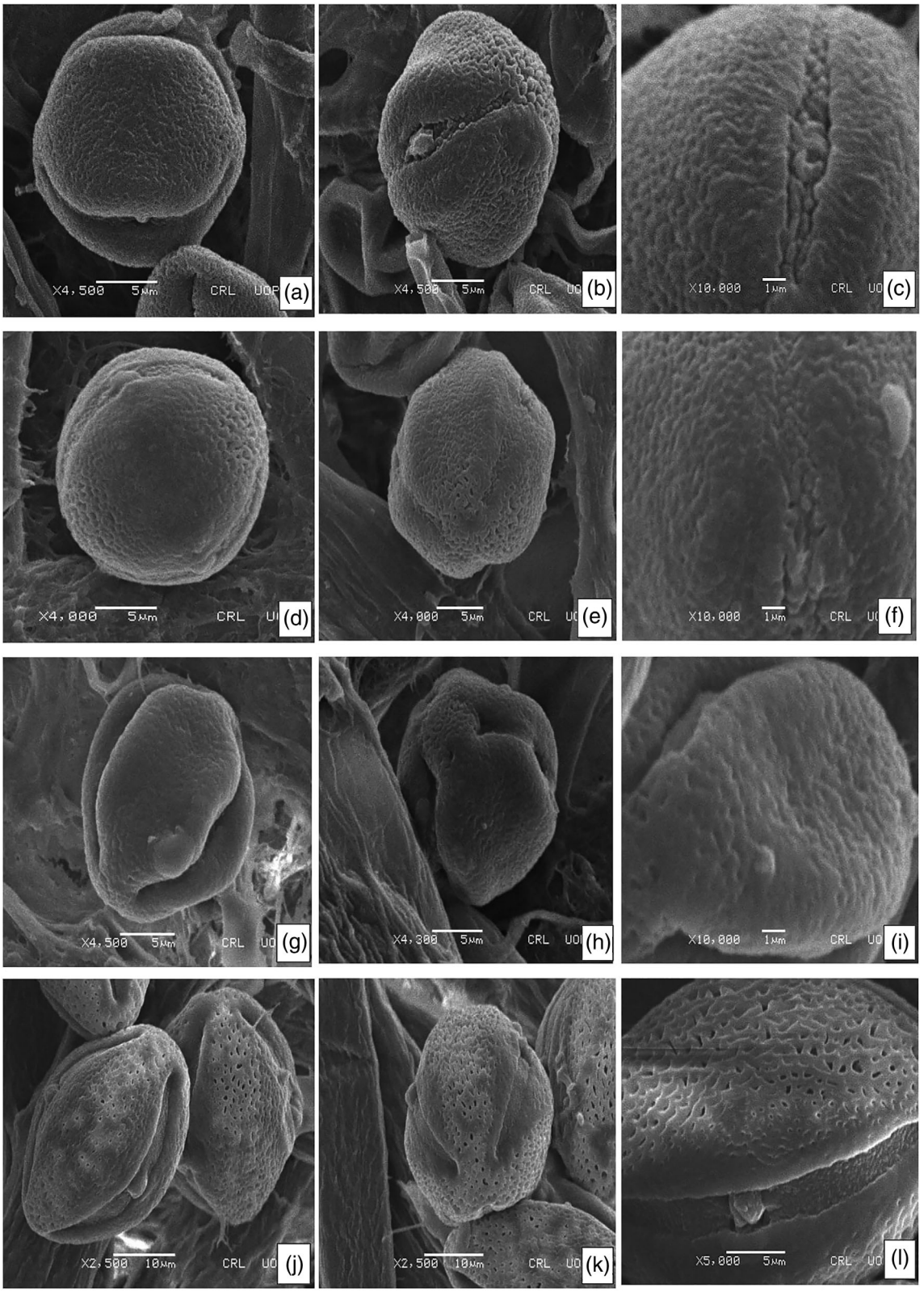

FIGURE 3 Scanning electron microscope microphotographs (SEM) of pollen grains; Vitis jacquemontii—(a) equatorial view, (b) polar view and extruded pore, (c) colpi and sculpturing; *Vitis vinifera*—(d) equatorial view, (e) polar view and extruded pore, (f) colpi and sculpturing; Vitis flexuosa—(g) equatorial view, (h) polar view and extruded pore, (i) exine sculpturing; *Parthenocissus quinquefolia*—(j) equatorial view, (k) polar view and extruded pore, (l) colpi and sculpturing

3.4 | *Parthenocissus quinquefolia* (L.) Planch.

Pollen is monad, medium sized, isopolar, circular in polar view, prolate-spheroidal in shape, and tricolporate with reticulate exine sculpturing. Average polar diameter is 30.51 ± 2.89 μm, equatorial diameter is 27.75 ± 1.36 μm, P/E ratio is 1.09 μm, exine thickness is 1.05 ± 0.11 μm, colpi length is 2.09 ± 2.09 μm, and width is 9.48 ± 2.91 μm (Figures 1k,l and 3j–l).

3.5 | *Parthenocissus semicordata* (Wall.) Planch.

Pollen grain is monad, medium sized, isopolar, circular in polar view, prolate-spheroidal in shape, and tricolpate with foveolate sculpturing. Average polar diameter is 28.83 ± 1.65 μm, equatorial diameter is 27.06 ± 1.2 μm, P/E ratio is 1.07 μm, exine thickness is 1.50 ± 0.1 μm, colpi length is 10.05 ± 2.17 μm, and width is 6.24 ± 1.92 μm (Figures 2b,c and 4a–c).

3.6 | *Parthenocissus tricuspidata* (Siebold & Zucc.) Planch.

Grains are monad, medium sized, isopolar, circular in polar view, prolate-spheroidal in shape, and tricolporate with perforate sculpturing. Average polar diameter is 29.94 ± 0.83 μm, equatorial diameter is 28.03 ± 1.08 μm, P/E ratio is 1.07 μm, exine thickness is 1.23 ± 0.09 μm, colpi length is 8.28 ± 2.97 μm, and width is 6.69 ± 1.43 μm (Figures 2e,f and 4d–f).

3.7 | *Ampelopsis vitifolia* subsp. *hazaraganjiensis* Nazim & Qaiser

Grains are monad, medium sized, isopolar, trigonal in polar view, subprolate in shape, and tricolpate with reticulate exine sculpturing. Average polar diameter is 30.43 ± 1.21 μm, equatorial diameter is 25.30 ± 0.09 μm, P/E ratio is 1.20 μm, exine thickness is 2.60 ± 0.20 μm, colpi length is 22.10 ± 0.90 μm, and width is 16.20 ± 2.10 μm (Figures 2h,i and 4g–i).

3.8 | *Cissus trifoliata* (L.) L.

Pollen grain is monad, medium sized, isopolar, trigonal in polar view, oblate-spheroidal in shape, and tricolporate with reticulate-perforate sculpturing. Average polar diameter is 24.19 ± 2.1 μm, equatorial diameter is 25.71 ± 2.52 μm, P/E ratio is 0.94 μm, exine thickness is 1.14 ± 0.35 μm, colpi length is 12.21 ± 2.44 μm, and width is 5.79 ± 2.31 μm (Figures 2k,l and 4j–l).

3.9 | *Cissus quadrangularis* L.

Pollen grain is monad, medium sized, isopolar, circular in polar view, oblate in shape, and tricolpate with reticulate-perforate sculpturing. Average polar diameter is 11.70 ± 0.15 μm, equatorial diameter is 17.30 ± 0.09 μm, P/E ratio is 0.67 μm, exine thickness is 2.01 ± 0.10 μm, colpi length is 12.30 ± 1.80 μm, and width is 7.20 ± 1.03 μm (Figures 2n,o and 4m–o).

4 | DISCUSSION

Palynology is the morphological characterization of pollen and spore. Palyno-morphological characteristics play a crucial role in the identification, classification, and species delimitation and serve as an additional tool for the systematic study of plant groups (Ashfaq et al., 2018; Khan et al., 2018).

The shape of pollen grain is an important character to differentiate the genera of Vitaceae (Najmaddin, 2014b). The dominant pollen shape was prolate to spheroidal observed in *Vitis vinifera*, *Vitis flexuosa*, *Parthenocissus quinquefolia*, *P. semicordata*, *P. tricuspidata* while *Vitis jacquemontii*, *Ampelopsis vitifolia*, *Cissus trifoliata*, and *Cissus quadrangularis* were observed with oblate-spheroidal, sub-prolate, oblate-spheroidal, and oblate shape, respectively. The studied plants displayed varied shape in polar view, ranged from circular or trigonal, except one species *Vitis vinifera* that has a circular-trigonal shape in polar view. Pollen grains were monad in terms of dispersal unit. The shape of pollen was determined based on the P/E ratio, ranging from 0.67 (*Cissus quadrangularis*) to 1.20 (*Ampelopsis vitifolia*) (Table 3). P/E may vary in different varieties of *Vitis vinifera* (Padureanu & Patras, 2018). The circular shape of *Vitis vinifera* determined in the present study is in accordance with the results of previous research work (Bonnefille, Melis, & Mussi, 2018).

The maximum exine thickness was noted in *Ampelopsis vitifolia* subsp. *hazaraganjiensis* (30.51 ± 2.89 μm), whereas minimum in *Parthenocissus quinquefolia* 1.05 ± 0.11 μm). There was a slight variation in terms of exine thickness. The colpi number was constant with three colpi in each, while length and width of colpi varied greatly. The maximum colpi length was recorded in *Ampelopsis vitifolia* subsp. *hazaraganjiensis* (22.10 ± 0.90 μm), whereas minimum in *Parthenocissus quinquefolia* (2.09 ± 2.09 μm).

Exine sculpturing features are significant taxonomically for the identification and species delimitation (Ullah et al., 2018). The sculpturing studied under the scanning electron microscope revealed slight variations among the species. Different types of sculpturing patterns were recorded including rugulate-reticulate (*Vitis jacquemontii*), reticulate (*Vitis vinifera*, *Parthenocissus quinquefolia*, *Ampelopsis vitifolia* subsp. *hazaraganjiensis*), perforate (*Parthenocissus tricuspidata*), foveolate (*Vitis flexuosa* and *Parthenocissus semicordata*), and reticulate-perforate (*Cissus trifoliata* and *Cissus quadrangularis*). Exine sculpturing characters have significant taxonomic potential for the identification and species delimitation. Pores were observed in *Vitis jacquemontii*, *V. vinifera*, *Parthenocissus quinquefolia*, *P. tricuspidata*, and *Cissus trifoliata*, while in rest of the species pores were absent. Slightly extruded pores of *Cissus quadrangularis* pollen were identified from the fossil sediments analyzed in the previous literature Gosling, Miller, & Livingstone, 2013). The present findings revealed the

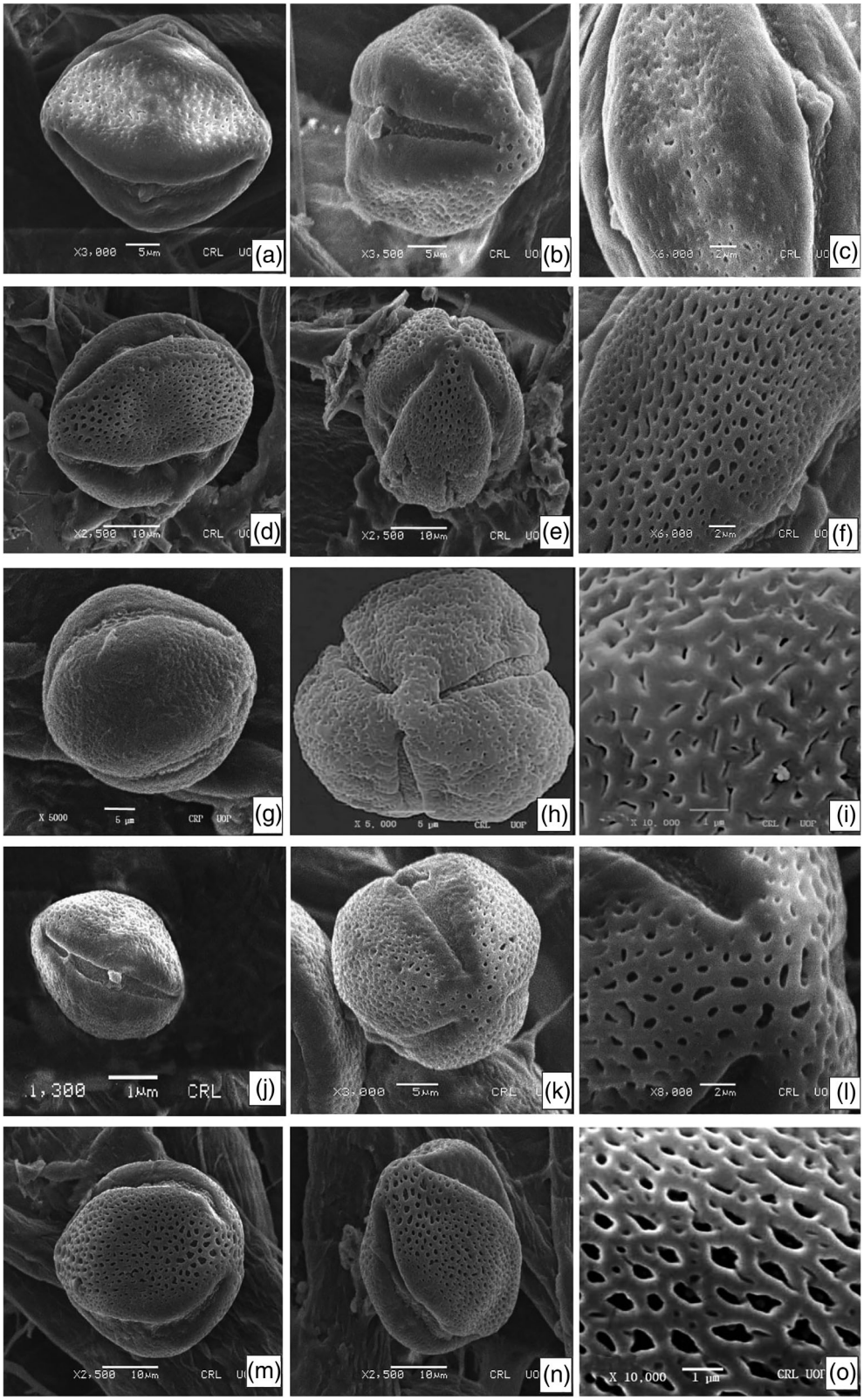

FIGURE 4 Scanning electron microscope microphotographs (SEM) of pollen grains; Parthenocissus semicordata—(a) equatorial view, (b) polar view (c) exine sculpturing; *Parthenocissus tricuspidata*—(d) equatorial view, (e) polar view, (f) exine sculpturing; Ampelopsis vitifolia subsp. hazaraganjiensis—(g) equatorial view, (h) polar view (i) exine sculpturing; *Cissus trifoliata*—(j) equatorial view, (k) polar view and extruded pore, (l) exine sculpturing; *Cissus quadrangularis* L. (m) equatorial view, (n) polar view, (o) exine thickness

reticulate type of sculpturing observed in *Parthenocissus quinquefolia*, which is in agreement with the observations of previous workers (Najmaddin, 2014a). The results of the present study are in accordance with the findings of Perveen and Qaiser (2008).

Pollen grains from male flower of *Vitis vinifera* var. *Baidhawi* were prolate spheroidal and from female flowers of *Vitis vinifera* var. *Tre rash*, Rash meri were spheroidal to oval and unaperturate. Colpi were absent (Najmaddin, Hussin, & Maideen, 2011). The extension of

developmental studies beyond what is currently known for Vitaceae, the morphological and anatomical and palynological characters will allow for a more stable classification of the family and advance the evolutionary aspects of Vitaceae and Rosids as a whole (Ickert-Bond, Gerrath, Posluszny, & Wen, 2015). Thus, it remains important to further test the family at the deep and shallow levels along with more robust phylogenomic data and advanced analytical tools to better understand the phylogenetic relationship.

5 | CONCLUSION

The present research clarifies the importance of light and scanning microscopy for the correct identification and species delimitation of the studies species of Vitaceae based on pollen morphology. The present study reveals that palynological characters under SEM and LM are of important value for the identification of Vitaceae and have significant potential for taxonomists for the accurate identification. The results of the study clearly demonstrated the variations in palyno-morphological characters may help the taxonomists by providing a light and primary step to understand the diversity among this group, which may lead to correct identification, classification of the family, and advancement in phylogenetic tree.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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