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POLLEN MORPHOLOGY OF THE GENUS *MALUS* (ROSACEAE)*

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Abstract – Pollen morphology of some species of the genus *Malus* (Rosaceae) was investigated taxonomically. All species are characteristic by tricolpate pollens. Exine sculpturing is variable but is mostly striate. However, species may differ in degree of density of ridges and their orientation. The exine patterns in *M. trilobata* and *M. florentina* are very similar and were characterized by short and very branched ridges. These show clear differences from other species, which have pollen grains with long and unbranched or slightly branched ridges.

Keywords – Pollen morphology, plant systematics, *Malus*, Rosaceae

1. INTRODUCTION

The genus *Malus* Mill. belongs to the Rosaceae family, subfamily Maloideae. It is a north temperate genus of 25-35 species, which are difficult to circumscribe due to a lack of distinguishing characters, widespread hybridization, transportation by people, escape from cultivation, and introgression. These factors may blur taxonomic boundaries. Nomenclatural confusion also results from the naming as a species of variants known only in cultivation [1, 2]. Reporting 50 species in *Malus* by Phipps *et al*. [1] shows the discrepancy on the species boundaries in this genus.

Differences in size and surface ultrastructure of pollen grains are useful for distinguishing species of fruit trees. Thakur and Thakur [3] stated that the "pollen exine pattern is so genetically stable for the different species of drupe plants that it can be used for species identification". Hebda *et al*. [4] showed that exine sculpturing, aperture and aperture zone structure, grain shape, and grain size are all useful characters to distinguish genera and even species of rosaceous pollen. Fogle [5, 6] used the length and width of the pollen grain, depth of exine ridges and prominence of pits in the exine to distinguish peach, nectarine, plum, cherry, apricot, apple and pear. He reported that peach and nectarine have the largest pollen grains and their ridges tend to be shallow and longitudinally oriented with some curved patterns. Pollen morphology of the Rosaceae of western Canada was investigated by Hebda and Chinnappa [7]. They concluded that *Amelanchier alnifolia* pollen grains exhibit systematic geographic variability.

Previously, the pollen of some varieties of *M. domestica* Borkhausen was identified [6]. Shim *et al.* [8] distinguished 8 ornamental *Malus* spp. cultivars on the basis of their pollen morphology and showed that pollen grains of cv. Bioneer are intermediate between that of its parents *M. sargentii* Rehd. and cv. Hoppa. Xiang and Sheng [9] reported the pollen morphology of studied species of the genus *Malus*, but only included *M. coronaria* (Mill.) L. of section *Chloromeles*. They did not, however, investigate the pollen of *M. trilobata* (Poir.) Schneid., *M. fusca* (Rafin) Schneid. and *M. florentina* (Zucc.) Schneid., which are included in this study.

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2. MATERIAL AND METHODS

Plant specimens were collected by the author or have been sent by botanical gardens. Herbarium specimens of all the material studied were made and deposited in the herbarium of the World Museum Liverpool (LIV), United Kingdom (Table 1). Nineteen *Malus* species were examined in ultrastructural studies of pollen grains. Flowers with dehiscent anthers were collected from herbarium specimens. Small quantities of pollen of each species were sifted onto separate polished aluminium disk stubs covered with double-sided transparent tape, sputter-coated with 60% gold-palladium in a polaron E 5100 coater, and viewed in a Philips 501B scanning electron microscope at accelerating voltages of 7.2 and 15.KV [10]. The middle part of the exine of pollen grains was photographed at x10,000 and ten pollen grains were measured.

Table 1. Dimensions of pollen grains of *Malus* species

Taxon	Accession no.	Length (μm)		Width (μm)		Length/width ratio	
		range	X± SD	range	X± SD	range	X± SD
M. niedzwetzkyana	1973.11840K	49.6-52.2	50.81±1.01	21.4-24.4	23.19±1.14	2.02-2.36	2.20±0.13
M. domestica	1973.19912K	46.0-48.3	47.10±0.86	18.8-21.2	20.18±0.78	2.23-2.45	2.33±0.07
M. baccata	1974.4220E	45.4-49.8	47.88±1.45	22.9-25.8	24.40±1.01	1.78-2.12	1.96±0.09
M. sargentii	4433 NBG	50.6-53.3	52.35±0.75	23.2-25.5	24.72±0.94	2.04-2.25	2.12±0.07
M. *sieboldii*	1987.2240E	46.4-49.7	47.85±1.22	20.2-22.2	21.55±0.69	2.15-2.33	2.22±0.06
M. x floribunda	1966.5033E	46.0-51.6	48.54±1.95	21.9-23.5	22.63±0.61	1.98-2.37	2.15±0.01
M. fusca	4435.NBG	41.5-47.2	44.24±2.20	19.4-21.6	20.32±0.71	2.02-2.80	2.18±0.10
M. kansuenses	1938.1119E	41.4-46.8	44.19±1.63	18.1-20.4	19.15±0.76	2.03-2.45	2.31±0.12
M. transitoria	1986.1601K	38.1-47.4	43.35±2.59	18.6-21.7	20.20±0.98	1.76-2.35	2.15±2.15
M. prattii	1909.1013E	38.2-39.7	38.75±0.07	19.6-22.0	20.56±0.75	1.81-1.97	1.89±0.06
M. florentina	1966.5032E	39.9-43.8	42.06±1.43	22.0-24.8	23.60±0.86	1.65-1.90	1.78±0.07
M. angustifolia	1986.1556K	52.9-58.4	55.96±1.84	23.8-25.8	24.74±0.70	2.06-2.04	2.26±011
M. coronaria	1986.47713K	54.2-59.0	56.03±1.24	23.3-27.6	25.34±1.15	2.13-2.38	2.21±0.07
M. glaucescens	1986.8280K	54.2-61.2	57.69±2.27	23.3-28.5	26.64±1.69	1.97-2.34	2.17±2.34
M. ioensis var. *palmer*i	1986.8032K	50.0-56.7	52.64±1.94	19.9-24.8	22.43±1.30	2.20-2.54	2.35±0.09
M. lancifolia	1986.8281K	40.5-46.7	42.89±2.09	19.2-22.3	20.64±0.95	1.94-2.18	2.07±0.07
M. platycarpa	NFC	48.9-54.3	51.12±1.72	26.6-31.7	28.74±1.61	1.69-1.90	1.78±0.08
M. tschonoskii	4434NBG	44.2-49.1	46.10±1.51	20.6-22.9	21.94±0.88	1.99-2.21	2.10±0.07
M. trilobata	1969.17535K	44.7-48.5	46.78±1.34	20.6-23.7	22.26±1.02	1.93-2.22	2.10± 0.10

Abreviations: K, Royal Botanic Gardens Kew, London; E, Royal Botanic Gardens Edinburgh, NBG, Ness Botanic Gardens, Neston, Liverpool; NFC, Brogdale, National fruit collection; X mean; SD standard deviation

3. RESULTS

3. 1. General information

The shape of all the pollen grains examined was elliptical, tricolpate with three germinal furrows, each furrow extending almost the full length of the pollen grain (Figs. 1-3). The dimensions of the pollen grains of the species examined are presented in Table 1. Mean size of the pollen grains ranges from 38.75 μm to 57.69 μm in length and from 19.15 μm to 28.74 μm in width. The length/width ratios were from 1.78 to 2.35. All of the American species except *M. lancifolia* have very large pollen. The largest mean pollen length occurs in *M. glaucescens* (54.2-61.2μm), *M. angustifolia* (52.9-58.4μm) and *M. coronaria* (54.2-59.0μm), while the smallest average pollen length occurs in *M. prattii* (38.2-39.7μm). The largest average pollen width occurs in *M. glaucescens* (23.3-28.5μm), *M. coronaria* (23.3-27.6μm), *M. angustifolia* (23.8-25.8μm) and *M. sargentii* (23.2-25.5μm), and the smallest in *M. kansuensis* (18.1-20.4μm).

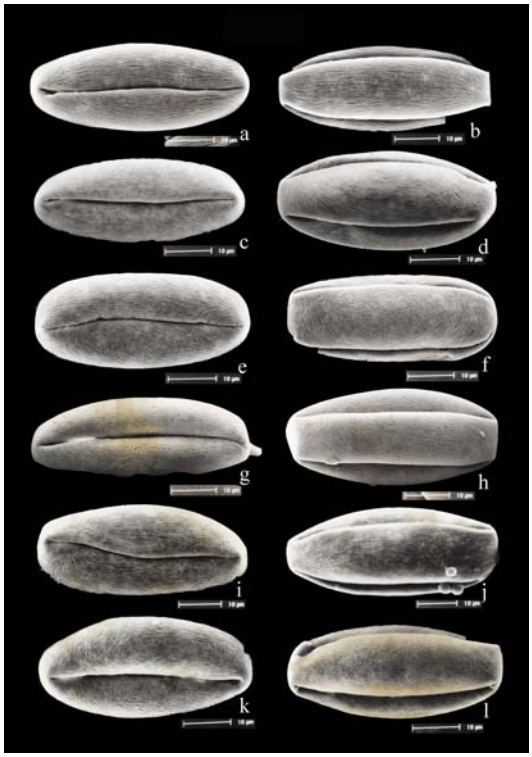

Fig. 1. Scanning electron micrograph of pollens of *Malus* species. a, b: *M. domestica* (a, side view; b, equatorial view); c, d: *M. niedzwetzkyana* (c, side view; d, equatorial view); e, f: *M baccata* (e, side view; f, equatorial view); g, h: *M. x floribunda* (g, side view; h, equatorial view); i, j: *M. sargentii* (i, side view; j, equatorial view); k, l: *M. sieboldii*; (k, side view; l, equatorial view)

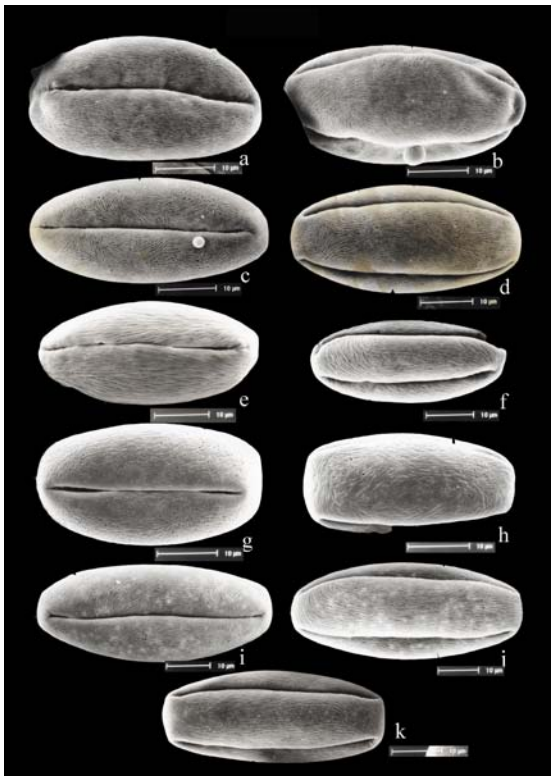

Fig. 2. Scanning electron micrograph of pollens of *Malus* species. a, b: *M. florentina* (a, side view; b, equatorial view); c, d: *M. fusca* (c, side view; d, equatorial view); e, f: *M. transitoria* (e, side view; f, equatorial view); g, h: *M. prattii* (g, side view; h, equatorial view); i, j: *M. angustifolia* (i, side view; j equatorial view); k: *M. kansuensis* (side view)

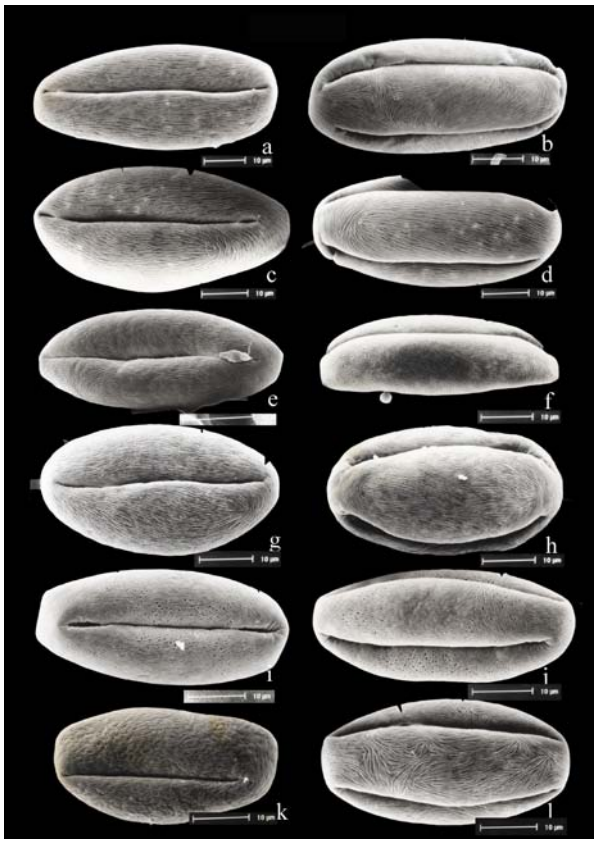

Fig. 3. Scanning electron micrograph of pollens of *Malus* species. a, b: *M. coronaria* (a, side view; b, equatorial view); c, d: *M. glaucescens* (c, side view; d, equatorial view); e, f: *M. ioensis* (e, side view; f, equatorial view); g, h: *M. platycarpa* (g, side view; h, equatorial view); i, j: *M. trilobata* (i, side view; j, equatorial view); k: *M. lancifolia* (side view); l: *M. tschonoskii* (equatorial view)

3. 2. Exine sculpture

The general pattern of the exine differs from species to species (Fig. 4). All the species examined have perforated exine surface except *M. coronaria*, *M. ioensis* var. *palmeri* and *M. lancifolia* (Fig. 4: m,o,p). Five different patterns have been recognized based on the ridge patterns on the surface of the exine including: smooth, parallel, curved, reticulate and tangled thread.

Among the species examined some are characterized by relatively smooth surfaces and not easily distinguishable ridges, such as *M. sieboldii* and *M. prattii* (Fig. 4: f,k), others by rough pollen grains with prominent ridges, among them *M. lancifolia* is the most conspicuous species with tangled thread decoration on the surface and very short, thick and confluent ridges (Fig. 4: p).

Pollen of the other species tends to have patterning which is longitudinally oriented, branched and sometimes with some of the ridges curved.

The spaces between the longitudinal ridges are wide in some species (e.g. *M. tschonoskii* (Fig. 4: q), *M. floribunda* (Fig. 4: d), *M. sargentii* (Fig. 4: e), *M. kansuensis* (Fig. 4: i), *M. baccata* (Fig. 4c), *M. niedzwetzkyana* (Fig. 4: b), *M. platycarpa* and *M. domestica* (Fig. 4: a)), while in other species they are very narrow and close. Examples are *M. ioensis* var. *palmeri* (Fig. 4: o), *M. trilobata* (Fig. 4: r) and *M. coronaria* (Fig. 4: m). However, in *M. coronaria*, *M. transitoria* and *M. fusca* both types could be seen in a single pollen grain (Figs. 4: m,j,h).

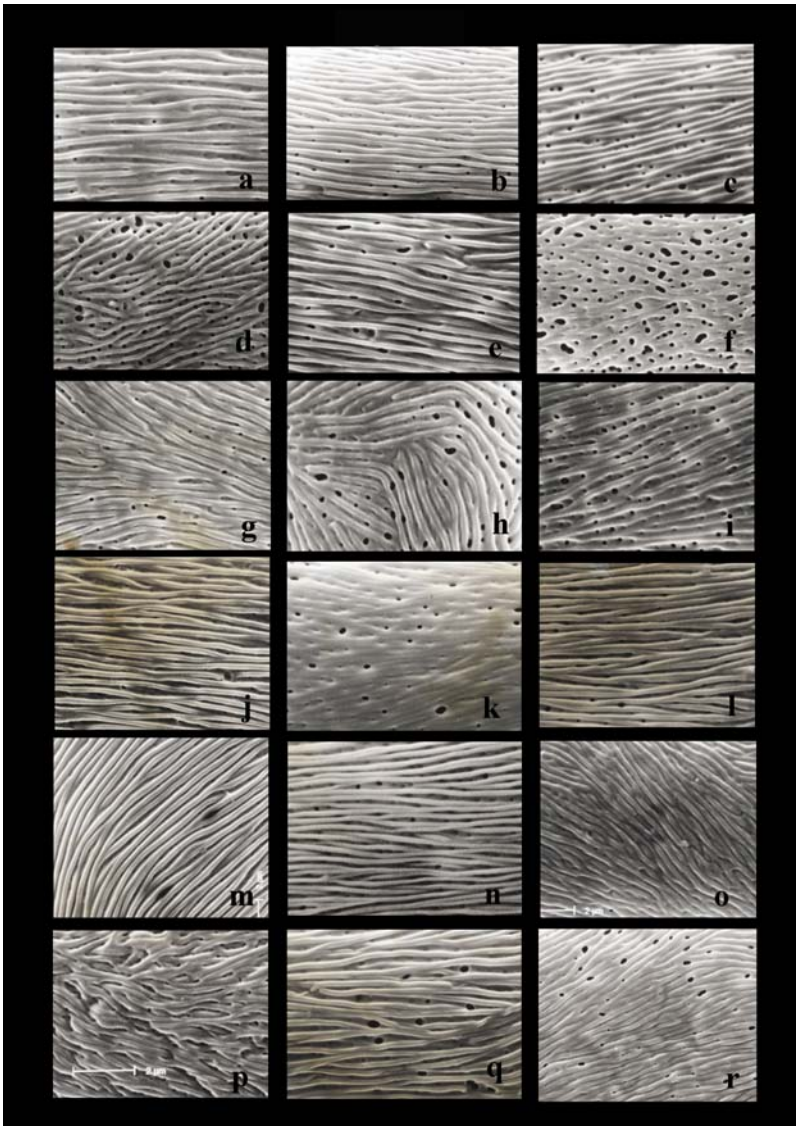

Fig. 4. Scanning electron micrograph of pollens of *Malus* species. Exine sculpture showing the ridges. a: *M. domestica;* b: *M. niedzwetzkyana;* c: *M. baccata*; d: *M. x floribunda*; e: *M. sargentii*; f: *M. sieboldii*; g: *M. florentina*; h: *M. fusca*; i: *M. kansuensis*; j: *M. transitoria*; k: *M. prattii*; l: *M. angustifolia*; m: *M. coronaria*; n: *M. glaucescens*; o: *M. ioensis*; p: *M. lancifolia*; q: *M. tschonoskii*; r: *M. trilobata*

3.3. Characteristics of sections and series of Malus and descriptions of pollen by species

The characters of the pollen morphology of sections and series are as follows:

3.3.1. Section Malus

3.3.1.1. Series Pumilae

Exine sculpture rough, perforated, striae parallel to the colpi, long, conjugate or curved at the poles.

3.3.1.1.1. M. domestica Borkhausen

Pollen grains 46.0-48.3 µm in length and 18.8-21.2 µm in width; surface rough, exine sculpture striate, ridges long, straight and parallel to the furrows, slightly curved at the ends; exine surface perforated and pits sparsely distributed (Fig. 1: a, b and Fig. 4: a).

3.3.1.1.2. M. niedzwetzkyana (Hemsl) Dieck

Pollen grains 49.6-52.2 µm in length and 21.4-24.4 µm in width; surface rough; exine sculpture parallel, straight and sometimes curved at the end of the grain; ridges far from each other, exine surface perforated and pits abundant (Fig. 1: c, d and Fig. 4: b).

3.3.1.2. Series Baccata

Exine sculpture smooth, striae parallel, curved at the poles, exine surface perforated.

3.3.1.2.1. M. baccata (L.) Borkh

Pollen grains 45.4-49.8 µm in length and 22.9-25.8 µmin width; pollen surface almost smooth; exine sculpture striate, short and occasionally branched, straight at the middle and curved at the ends; pits small (less than 0.12 µm), (Fig. 1: e, f and Fig. 4: c).

3.3.2. Section Sorbomalus

3.3.2.1. Series Sieboldianae

Strongly perforated, irregular, nearly reticulate (except *M. sargentii*) with parallel striae.

3.3.2.1.1. M. x floribunda Van Houtte

Pollen grains 46.0-51.6 µm in length and 21.0-23.5 µm in width; surface rough; exine sculpture striate, ridges far from each other, very branched; pits abundant and variable in sizes (Fig. 1: g, h and Fig. 4: d).

3.3.2.1.2. M. sargentii Rehd

Pollen grains 50.6-53.3 µm in length and 23.2-25.5 µm in width; surface rough; exine sculpture striate; ridges long, straight, sometimes curved at the ends or near the edge of furrows; pits sparsely distributed, elliptic or sometimes rounded (Fig. 1: i, j and Fig. 4: e).

3.3.2.1.3. M. sieboldii (Regel) Rehder

Pollen grains 46.4-49.7 µm in length and 20.2-22.2 µm in width; pollen surface smooth, exine sculpture reticulate; pits abundant and very large (0.24-0.29 µm), pit shape irregular (Fig. 1: k, l and Fig. 4: f).

3.3.2.2. Series Floreninae

3.3.2.2.1. M. florentina (Zucc.) Schneid

Pollen grains 39.9-43.8 µm in length and 22.0-24.8 µm in width; surface rough, exine sculpture striate, not very straight and slightly curved at the ends. Ridges short and very branched, pits small (0.12-0.24 µm), (Fig. 2: a, b and Fig. 4: g).

3.3.2.3. Series Kansuenses

Striae regular, parallel to colpi, not conjugate, but curved at the poles (except *M. fusca*)

3.3.2.3.1. M. fusca (Rafin.) Schneid

Pollen grains 41.5-47.2 µm in length and 19.4-21.6 µm in width; surface rough, exine sculpture striate, straight and curved, both types occurring on each pollen grain; pits present, especially abundant in

area of curved ridges (Fig. 2: c, d and Fig. 4: h).

3.3.2.3.2. M. kansuensis (Batal.) Schneid

Pollen grains 41.4-46.8 µm in length and 18.1-20.4 µm in width; surface rough, striae straight and nearly parallel to the colpi at the middle of the grain and curved at the ends, far from each other, pits abundant (Fig. 2: k and Fig. 4: i).

3.3.2.3.3. M. transitoria (Batal.) Schneid

Pollen grains 38.1-47.4 µm in length and 18.6-21.7 µm in width; surface rough, exine sculpture striate, mostly straight and parallel to the colpi, sometimes curved at the ends; ridges long and branched, close to each other; pits very rare and small at the middle and more numerous at the poles (Fig. 2: e, f and Fig. 4: j).

3.3.2.4. Series Yunanenses

Exine sculpture smooth, striae irregular, perforation present.

3.3.2.4.1. M. prattii (Hemsel.) Schneid

Pollen grains 38.2-39.7 µm in length and 19.6-22.0 µm in width; surface smooth, exine sculpture striate, irregular, ridges very close to each other usually with two or three fused to make a compound wide ridge; pits abundant, large and round, although Xiang and Sheng [9] reported that pits are absent in *M. prattii*, distinct from other species by very smooth and fused ridges (Fig 2: g, h and Fig. 4: k).

3.3.3. Section Chloromeles

Exine sculpture rough, striae very variable in the species*; M. ioensis*, *M. platycarpa* and *M. lancifolia* have irregular striae; *M. coronaria*, *M. glaucescens* and *M. angustifolia* have regular and parallel striae.

3.3.3.1. M. angustifolia (Ait.) Michx

Pollen grains 52.58-58.4 µm in length and 23.8-25.8 µm in width; pollen surface rough, exine sculpture striate; ridges long, parallel with furrows and curved at the ends; pits mostly at the poles, sparse at the middle of the grain (Fig. 2:g, h and Fig. 4: l).

3.3.3.2. M. coronaria (L.) Mill

Pollen grains 54.2-59.0 µm in length and 23.3-27.6 µm in width; surface rough, exine sculpture slightly striate and mostly parallels, rarely branched sometimes curved and may be different in each part of a pollen grain; pits rare, especially when the ridges are curved (Fig. 3: a, b and Fig. 4: m).

3.3.3.3. M. glaucescens Rehd

Pollen grains 54.2-61.2 µm in length and 23.3-28.5 µm in width; surface rough, exine sculpture striate mostly unbranched and close to each other; ridges parallels to the furrows and curved at the ends, far from each other; pits sparsely distributed and round (Fig. 3: c, d and Fig. 4:n).

3.3.3.4. M. ioensis (Wood) Brit

Pollen grains 50.0-56.7 µm in length and 19.9-24.8 µm in width; ridges close in the middle and far apart at the poles and edge of the furrows. Very similar to *M. trilobata* in exine sculpture but can be distinguished by the absence of pits (Fig. 3: e, f and Fig. 4: o).

3.3.3.5. M. lancifolia Rehd

Pollen grains 40.5-46.7 µm in length and 19.2-22.3 µm in width; surface rough, exine sculpture striate and tangled thread; ridges very short; pits very few. This species can easily be distinguished by the tangled thread sculpture (Fig. 3: k and Fig. 4: p).

3.3.3.6. M. platycarpa Rehd

Pollen grains 48.9-54.3 µm in length and 26.6-.31.7 µm in width; surface rough, exine sculpture striate, striae mostly unbranched and close and merging into each other; pits absent in the middle and very rare at the poles. The pollen of this species is very distinct in its greater width than any other studied species (Fig. 3: g, h).

3.3.4. Section Docyniopsis

Exine sculpture rough; striae irregular; ridges very thick.

3.3.4.1. M. tschonoskii (Maxim) Schneid

Pollen grains 44.2-49.1 µm in length and 20.6-22.9 µm in width; surface very rough, exine sculpture striate; ridges very thick, far from each other, both kinds of striate (straight and curved) could be seen. This species is easily distinguishable by the very thick ridges (Fig. 3: l and Fig. 4: q).

3.3.5. Section Eriolobus

Striae very dense and smooth in the middle, well separated at the poles and near colpi.

3.3.5.1. M. trilobata (Poir.) Schneid

Pollen grains 44.7-48.5 µm in length and 20.6-23.7 µm in width; surface rough, exine sculpture striate with short and branched ridges; ridges far from each other except in the middle of the pollen grains where the ridges are very close to each other and look smooth; pits are elliptic and scattered all over the surface. However, they are more distinct at the ends and edges of the furrows. All the grains show the same patterns in each lobe (Fig. 3: i, g and Fig. 4: r).

4. DISCUSSION

The significant variation found on pollen grains of *Malus* suggests the potential taxonomic value of pollen characteristics. The results obtained in this study support the taxonomic classification of the genus. In all of the species examined, tricolpate pollen is characteristic without any exceptions. Exine sculpturing is not homogenous. Variation in exine sculpture was observed in the pollen of all taxa. However, the most constant part is the middle of each pollen grain. Exine sculpturing is variable, but mostly striate. However, species may differ in the degree of density of ridges and their orientation. The ridges may be densely packed with obscured perforations, or they may be widely spaced resulting in reticuloid patterns. Ridges also vary from long and parallel to short and irregular.

The mean size of pollen grains shows considerable variation among species (Table 1). Species of the series *Chloromeles*, *Pumilae* and *M. sargentii* of the series *Sieboldianae* have the largest pollen grain. The significance of the pollen grain size is uncertain as diploids, tetraploids and sometimes triploids have also been recorded within some species and pollen size is often closely linked with ploidy level [11].

Variation within the species of the series is observed in series *Sieboldianae*, *Kansuenses* and *Chloromeles*. In series *Sieboldianae*, *M. sargentii* shows variation from other species with regular striae

and reduction in perforation. *M. fusca* of series *Kansuenses* is rather different from other *Kansuenses* with ridges parallel to the colpi. *M. fusca* has irregular striae which curve at the margins of the colpi.

M. trilobata and *M. florentina* are very similar to each other and to *Chloromeles* in having few pits and dense ridges which distinguish these taxa from other species in the genus.

The evolution of exine sculpture may be from rough and dense ridges with small and few pits in *Chloromeles* to smooth or sparse ridges and large and more numerous pits in *Sieboldianae*. In this case series *Yunnanenses*, with smooth and sparse ridges and a perforated surface may be considered derivative, but this is not in accordance with the postulated evolutionary sequence of morphological characteristics in which *Yunnanenses* are considered a primitive taxon. Considering 11 morphological characters, Yunnanenses showed no apomorphic characters [12]. Similar exine patterns in *M. trilobata* and *M. florentina* and dissimilarity from others are in line with other similarities between these species (including lobed leaves, white petals, intermediate number of flower per inflorescence, intermediate carpel connation and small fruits, and also the same geographical distribution in the Mediterranean area) [12] and their isolated position in the genus. Similarities between these two species and *M. tschonoskii* may be the persistence of an ancestral condition.

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