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The Significance of Pollen Morphology in the Taxonomy of the Genus *Durio* (Bombacaceae)

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Abstract

The pollen grains of 20 *Durio* species and 5 clones of *D. zibethinus* were studied. Based on the exine sculpture of the pollen, the *Durio* species can be divided into three major groups, i.e. psilate, scabrate and verrucate. However, variation in the exine sculpture is also exhibited in *D. zibethinus*, *D. malaccensis* and *D. singaporensis*. In addition, pollen size and wall thickness provide useful taxonomic evidence in distinguishing certain species. Pollen characters, however, could be used as supplementary characters in the identification of *Durio* species.

Introduction

The taxonomic value of palynology is well-established (Erdtman 1952, Sporne 1972 and Ferguson, 1979) and pollen morphology of the Bombacaceae has been investigated by several workers. Erdtman (1952) described the pollen morphology of two species (*Durio oblongus* and *D. testudinarum*), Chaudhuri (1965) studied the pollen of four species (*D. griffithii*, *D. pinangianus*, *D. singaporensis* and *D. testudinarum*) and Sharma (1970) seven *Durio* species (the previous four as well as *D. lowianus*, *D. carinatus* and *D. oblongus*). Sharma confirmed that pollen of the Bombacaceae is eurypalynous and indicates relatedness to the Malvaceae, Sterculaceae and Tiliaceae. He concluded that pollen characteristics provide an important basis for generic and specific delimitations but this must be assessed together with other morphological data. Nilsson and Robyns (1986), who studied the pollen of nine *Durio* species using scanning electron microscopy, described *Durio* types as heterogenous. Three of these species *D. acutifolius*, *D. graveolens* and *D. zibethinus* had not previously been studied. The pollen of the nine species were grouped under the *Durio*-type, which is characterised by being more or less smooth tectate-perforate, commonly with aspidote pollen grains with short, compound, often transitional apertures. Abang Mokhtar (1991), who studied the pollen of 16 *Durio* species from Sarawak, distinguished two pollen types in *Durio*, the psilate and verrucose types. However, he did not find significant diagnostic differences in the general morphology of the pollen characters

that could be used to identify the species. He concluded that pollen morphology alone has little value in solving the taxonomic problems associated with the Sarawak species.

The aim of this study is to investigate the pollen of 20 *Durio* species in Malaysia and to assess whether pollen characters can contribute taxonomic evidence.

Materials and Methods

Pollen acetolysis was carried out on 20 *Durio* species and 5 clones of *D. zibethinus*. Pollen was collected from herbarium specimens as well as from fresh flowers collected in the field (Table 1). Dried flowers were first boiled for about 5 min and left to cool before removing the anthers. Pollen was acetolysed (Erdtman, 1952) and the sample was divided into two parts; one for examination with the light microscope and the other for scanning electron microscopy.

Table 1: List of *Durio* species and specimens examined

Species		Herbarium specimen	Fresh specimen (Locality)
1.	*D. affinis* Becc.	KEP 3945	
2.	*D. carinatus* Mast.	FRI 3819	Rompin
3.	*D. dulcis* Becc.	SAN 15389	
4.	*D. excelsus* (Korth.) Bakh.	A 13513	
5.	*D. grandiflorus* (Mast.) Kosterm. et Soeg.	SAN 4611	
6.	*D. graveolens* Becc.	KEP 65525	Jerangau
7a.	*D. griffithii* (Mast.) Bakh.	KEP 7344	Semenyih
7b.	*D. griffithii* var. *acutifolius* Salma var. nov. (in ms.)	SAN 34716	
8.	*D. johoricus* Salma, sp. nov. (in ms.)	MS 1674	
9.	*D. kinabaluensis* (Bakh. ex Wyatt-Smith) Kosterm. et Soeg.	SAN 105554	
10.	*D. kutejensis* (Hassk.) Becc.	S 22848	
11.	*D. lanceolatus* Mast.	SAN 15373	
12.	*D. lowianus* Scort. ex King		Serdang
13.	*D. macrophyllus* (King) Ridl.	KEP 2731	
14.	*D. malaccensis* Planch. ex Mast.	KEP 64878	Semenyih
15.	*D. oxleyanus* Griff.	FRI 21576	Jerangau
16.	*D. pinangianus* (Becc.) Ridl.	KEP 3780	
17a.	*D. singaporensis* Ridl.	FRI 20236	Jerangau
17b.	*D. singaporensis* var. *jerangauensis* Salma var. nov. (in ms.)	MAR 6529	Jerangau
18.	*D. testudinarum* Becc.	A 3101	
19.	*D. wyatt-smithii* Kosterm.	KEP 57451	Bt. Bauk, Dungun
20.	*D. zibethinus* Murr. (Clones D24, Kop, KK2, D111, D88)		Serdang

Light Microscope Slide Preparation

Glycerol (50%) was added to the acetolysed pollen and stirred well before centrifuging for about 10 min. The glycerol was then decanted and the tubes were inverted overnight at room temperature to drain off excess solution. The dried pollen was placed in a drop of glycerine jelly stained with safranin on a clean, labelled slide. A coverslip was lowered carefully and gently on to the sample to avoid the formation of air bubbles. The slides were placed on a hot plate at 60°C to spread the glycerine jelly. The coverslip was sealed with clear nail varnish. Measurements (Table 2) were taken on 20 pollen grains for each sample using a calibrated eyepiece graticule.

Table 2. Pollen Morphological Characters of *Durio* Species

Characters Species		Pollen shape	Exine sculpture	Polar diameter µm	Equatorial diameter µm	P/E.100	Wall thickness µm
1.	*D. affinis*	prolate spheroidal	psilate	(92—)100.5(—112)	(92—)93.7(—98)	107	(2.0—)3.4(—4.0)
2.	*D. carinatus*	prolate spheroidal	scabrate	(52—)80(—100)	(72—)74(—76)	108	(2.0—)3.3(—4.0)
3.	*D. dulcis*	prolate spheroidal	psilate	(88—)89.4(—90)	(76—)80(—84)	109	(2.0—)3.4(—4.0)
4.	*D. excelsus*	oblate spheroidal	psilate	(52—)66(—80)	(60—)73.5(—82)	99	(0.8—)1.7(—2.0)
5.	*D. grandiflorus*	oblate spheroidal	psilate	(68—)81.4(—100)	(76—)84(—100)	97	(2.0—)3.4(—4.0)
6.	*D. graveolens*	prolate spheroidal;	psilate	(68—)72(—88)	(70—)70.8(—80)	102	(3.0—)3.8(—4.0)
		subprolate	psilate	(72—)88(—96)	(60—)66(—72)	133	(3.2—)3.4(—3.6)
7a.	*D. griffithii*	oblate spheroidal	psilate perforate	(46—)53.5(—60)	(52—)58.5(—62)	92	(1.2—)1.8(—2.0)
7b.	*D. griffithii* var. *acutifolius*	oblate spheroidal	psilate	(46—)51(—56)	(50—)54.5(—58)	94	(1.2—)1.6(—2.0)
8.	*D. johoricus*	prolate spheroidal	scabrate	(80—)88.9(—100)	(72—)81(—92)	110	(2.4—)3.7(—4.0)
9.	*D. kinabaluensis*	prolate spheroidal	scabrate	(80—)81.5(—84)	(72—)75.7(—80)	108	(2.4—)3.1(—3.6)
10.	*D. kutejensis*	prolate spheroidal	psilate	(80—)83.5(—88)	(72—)76(—80)	110	(2.8—)3.5(—4.0)
11.	*D. lanceolatus*	subprolate	scabrate	(66—)70.8(—76)	(50—)68.7(—80)	115	(3.6—)3.9(—4.0)

Table 2 (Cont.)

Characters Species		Pollen shape	Exine sculpture	Polar diameter µm	Equatorial diameter µm	P/E.100	Wall thickness µm
12.	*D. lowianus*	Oblate spheroidal;	psilate	(72—)74.6(-80)	(72—)78(—82)	96	(2.8—)3.5(—4.0)
		prolate spheroidal	psilate	(50-)71.3(—80)	(64—)70.3(—88)	101	(3.2—)3.5(—4.0)
13.	*D. macrophyllus*	prolate spheroidal	psilate	(76—)83.8(—96)	(80—)82(—92)	102	(3.2—)3.8(—4.0)
14.	*D. maluccensis*	prolate spheroidal;	psilate rarely verrucose;	(76—)80(—92)	(72—)74.4(—80)	108	(2.8—)3.1 (—3.2)
		prolate spheroidal	psilate	(72—)76.4(—80)	(56—)68(—76)	113	(3.2—)3.7(—4.0)
15.	*D. oxleyanus*	oblate spheroidal	scabrate	(52—)55.1(—56)	(52—)53.7(—56)	96	(2.0—)2.8(—3.0)
16.	*D. pinangianus*	oblate spheroidal	psilate	(88—)98(—108)	(92—)104(—120)	94	(3.2—)3.7(—4.0)
17.	*D. singaporensis*	prolate	scabrate	(80—)94(—104)	(80—)81.5(—84)	116	(3.2—)3.9(—4.0)
17b.	*D. singaporensis* var. *jerangauensis*	prolate	psilate	(76—)88(—100)	(68—)77.3(—84)	114	(3.2—)3.9(—4.0)
18.	*D. testudinarum*	prolate	verrucate	(92—)109(—145)	(68—)81.6(—92)	134	(4.0—)5.0(—8.0)
19.	*D. wyatt smithii*	prolate	psilate	(76—)84(—92)	(64—)76.7(—84)	110	(3.6—)3.9(—4.0)
20.	*D. zibethinus* (D24)	oblate spheroidal	psilate	(88—)90(—92)	(92—)94(—96)	95	(3.6—)3.9(—4.0)
	D88	subprolate	scabrate	(104—)106(—108)	(88—)90(—92)	117	(3.4-)3.8(—4.0)
	D11	subprolate	scabrate	(112—)118(—128)	(92—)95(—100)	124	(3.6—)3.8(—4.0)
	KK2	subprolate	scabrate	(96—)109(—120)	(88—)92(—96)	118	(3.6—)3.9(—4.0)
	Kop	prolate spheroidal	scabrate	(80—)92(—104)	(84—)89(—96)	103	(3.4—)3.8(—4.0)

Scanning Electron Microscope Preparation

The pollen sample was dehydrated through a series of 50%, 70%, 90% and absolute alcohol. A small square of double-sided sticky tape was stuck on to a labelled SEM stub. The pollen sample was sucked using a capillary tube and placed onto the stub. Each specimen was placed on a different stub. The stubs were coated with gold at 30 nm. The samples were then scanned using the JEOL JSM - 35C scanning electron microscope at Universiti Putra Malaysia, Serdang. Photomicrographs were taken (Plate 1) and features of the pollen grains recorded (Table 2).

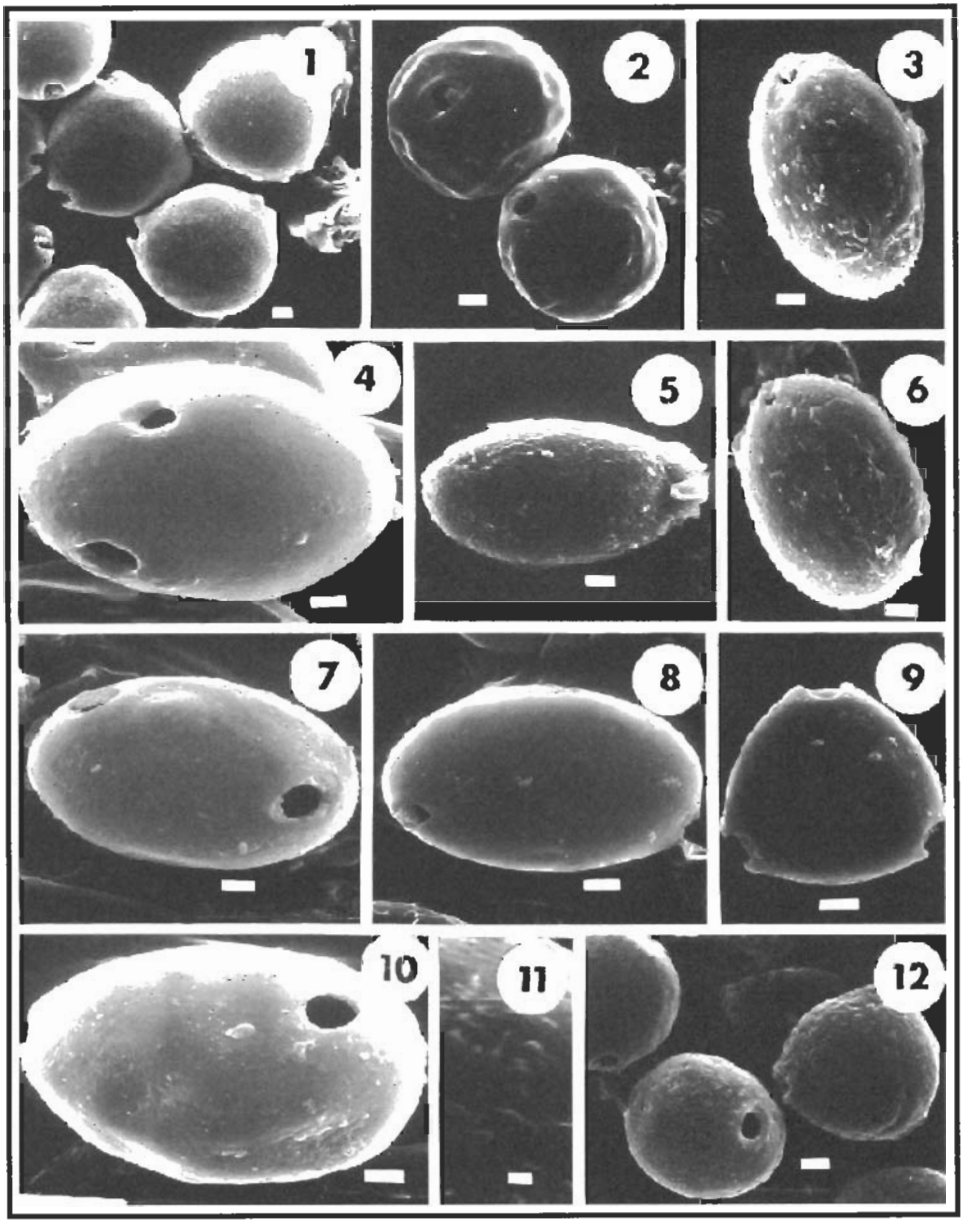

Plate 1. SEM micrographs showing pollen grain shapes and surface sculpturing in *Durio* species. 1. *D. affinis*; 2. *D. pinangianus*; 3. *D. lowianus*; 4. *D. kutejensis*; 5. *D. oxleyanus*; 6. *D. carinatus;* 7. *D. grandiflorus*; 8. *D. lanceolatus*; 9. *D. graveolens*; 10. *D. dulcis*; 11 & 12. *D. macrophyllus*. Scale bars: 1--10 & 12 = 10 µm, 11= 1 µm

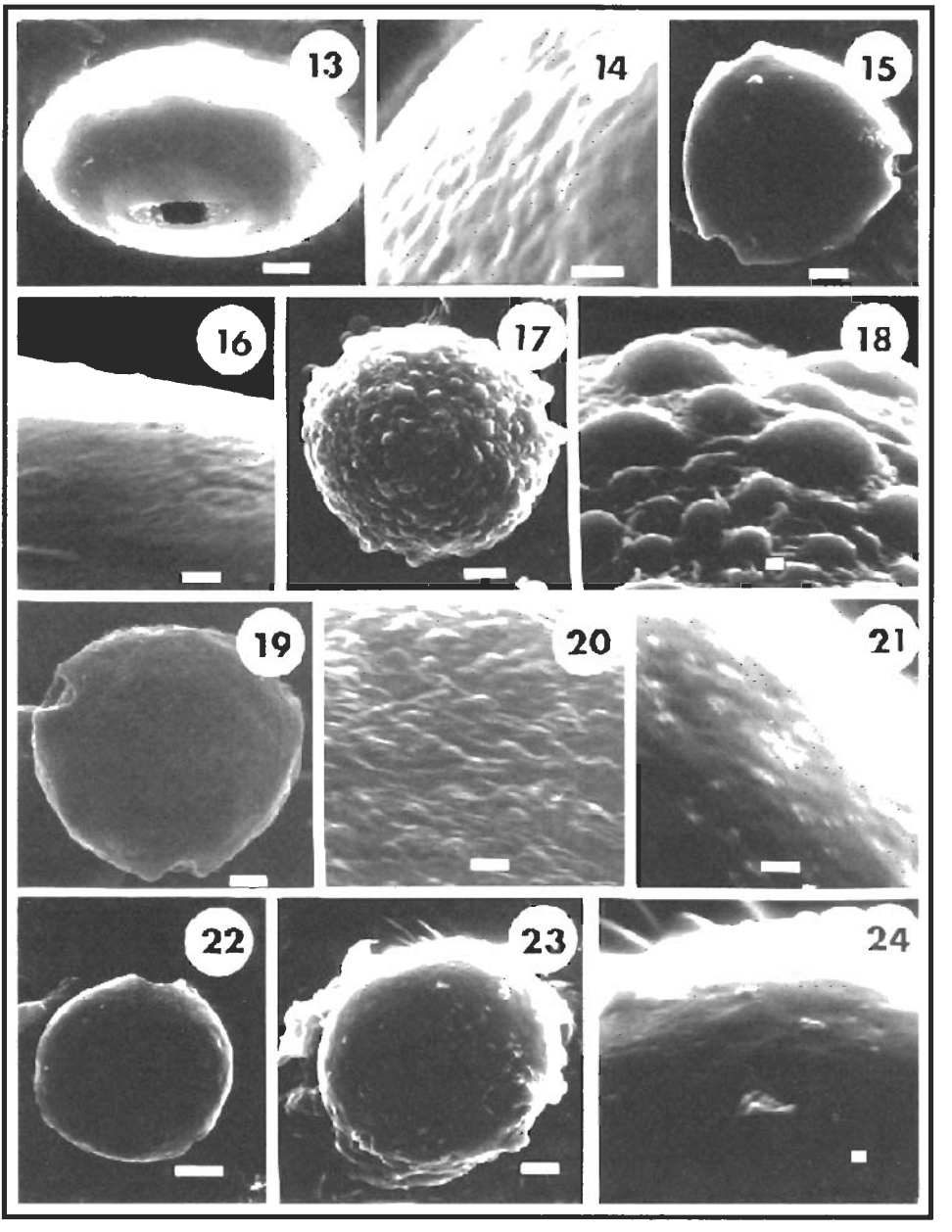

Plate 1(cont.). SEM micrographs showing pollen grain shapes and surface sculpturing in *Durio* species. 13 & 14. *D. excelsus*; 15 &16. *D. wyatt-smithii*; 17 & 18. *D. testudinarum*; 19 & 20. *D. singaporensis*; 21 & 22. *D. griffithii*; 23 & 24. *D. johoricus*. Scale bars: 13, 15, 17, 19, 22 & 23 = 10 µm. 14, 16, 18, 20, 21& 24 = 1 µm

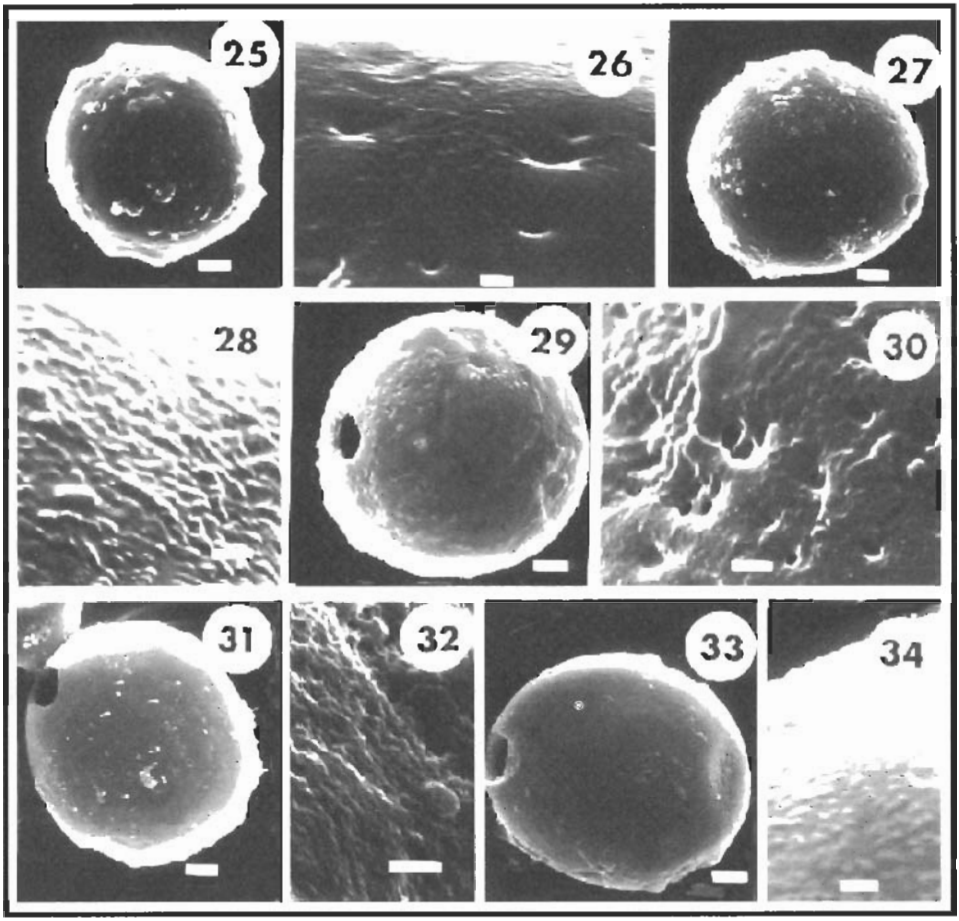

Plate1(cont.). SEM micrographs showing pollen grain shapes and surface sculpturing in *D. zibethinus* clones. 25 & 26. D24; 27 & 28. Kop; 29 & 30. KK2; 31 & 32. D111; 33 & 34. D88. Scale bars: 25, 27, 29, 31, 33 = 10 μm. 26, 28, 30, 32, 34 = 1 μm.

Results and Discussion

The photomicrographs of pollen using SEM are shown in Plate 1. Table 2 records the morphological characters of the pollen.

Characters, such as pollen grain shape, size, and exine sculpture, varied between species (Table 2). Based on exine ornamentation, *Durio* pollen could be divided into three groups i.e. verrucose, scabrate and psilate. However, Abang Mokhtar (1991) divided the pollen into only two groups, verrucose and psilate (this latter included microreticulate to finely tectate perforate).

Verrucose pollen was found only in *D. testudinarum* (Plates 1.17 and 1.18). Nilsson and Robyns (1986) and Abang Mokhtar (1991) also described *D. testudinarum* as having verrucose exine.

The scabrate exine was found in *D. carinatus*, *D. johoricus*, *D. lanceolatus*, *D. oxleyanus*, *D. singaporensis*, and D88, D111, KK2 and Kop clones of *D. zibethinus* (Plates 1.6, 1.23 & 1.24, 1.8, 1.5, 1.19 & 1.20; 1.33 & 1.34, 1.31 & 1.32, 1.29 & 1.30, 1.27 & 1.28, respectively). Sharma (1970) had also observed the finely scabrate exine of *D. carinatus* and *D. lowianus*. However, Abang Mokhtar (1991) reported that pollen of *D. dulcis*, *D. lanceolatus*, *D. carinatus* and *D. grandiflorus* was psilate.

Other species, such as *D. affinis*, *D. dulcis*, *D. excelsus*, *D. grandiflorus*, *D. graveolens*, *D. griffithii*, *D. kutejensis*, *D. lowianus*, *D. macrophyllus*, , *D. pinangianus*, *D. wyatt-smithii* and *D. zibethinus* (clone D24) possessed pollen with psilate exine sculpturing (Plates 1.1, 1.10, 1.13 & 1.14, 1.7, 1.9, 1.21 & 1.22, 1.4, 1.3, 1.11 & 1.12, 1.2, 1.15 & 1.16 and 1.25 & 1.26). Similarly, Abang Mokhtar (1991) observed the psilate exine sculpture in *D. kutejensis* pollen. However, Sharma (1970) reported exine sculpture of *D. griffithii* and *D. pinangianus* as scabrate, while Abang Mokhtar (1991) reported the exine of *D. affinis* and *D. graveolens* as verrucose.

Perforation occurred on the exine of some *Durio* species. In *D. griffithii* (Plates 1.21 & 1.22) and clones D 24 (Plates 1.25 & 1.26) and KK2 (Plates 1.29 & 1.30) of *D. zibethinus*, the exine was perforated as well. Nilsson and Robyns (1986) reported that the exine of *D. carinatus*, one collection of *D. graveolens* and *D. singaporensis* had few tectal perforations. In fact these species have similar exine sculpture but are described differently by different workers.

Variation in exine sculpture can occur within a species as was seen in *D. zibethinus*, where the exine varied from psilate to scabrate (Plates 1.25—1.34). Similarly for species such as *D. graveolens*, the exine sculpture varied from sparsely tectal perforate to granulate and verrucate. A similar observation was reported by Nilsson and Robyns (1986). However, variation in exine sculpture within *Durio* species had not been reported by Sharma (1970) and Abang Mokhtar (1991). Since variation in exine sculpture occurs between different clones of *D. zibethinus*, it is probable that a similar situation occurs in *D. graveolens*, *D. lowianus* and *D. malaccensis* because these species are also very variable based on fruit morphological characters.

Based on the classification of pollen shape by Erdtman (1952), the shape of *Durio* pollen varied from oblate spheroidal to prolate spheroidal, subprolate and prolate. Abang Mokhtar (1991) found a similar range of pollen shape in *D. carinatus*, *D. griffithii* var. *acutifolius*, *D. excelsus* and D. *grandiflorus*, while other species, such as *D. affinis*, *D. dulcis*, *D.*

graveolens, *D. kutejensis*, *D. oxleyanus*, were reported as oblate spheroidal. Sharma (1970) showed *D. lowianus* and *D. pinangianus* had a similar pollen shape, but that pollen of *D. griffithii* and *D. singaporensis* were oblate and that of *D. carinatus* oblate spheroidal.

Generally, in the majority of species the thickness of the pollen wall varied from 2—4 mm. However, the pollen wall of *D. griffithii* and *D. excelsus* was thin (less than 2 mm) while that of *D. testudinarum* was thick (greater than 4 mm).

Size varied from 46 to 145 mm for the polar diameter (P) and 50 to 120 mm for the equatorial diameter (E) (Table 2). According to the classification of the pollen size by Erdtman (1952), *Durio* pollen can be divided into three groups, i.e. medium (25—50 mm), large (50—100 mm) and very large (100—200 mm). Most *Durio* species fell within the large group except for *D. griffithii*, which had medium to large pollen, and *D. affinis*, *D. oxleyanus*, *D. pinangianus*, *D. testudinarum* and *D. zibethinus*, which possessed large to very large pollen (Table 2). Similar results were obtained by Abang Mokhtar (1991), except that pollen of his sample of *D. griffithii* and *D. acutifolius* fell within the medium-sized group and *D. graveolens* in the very large group. This difference in size was probably due to whether herbarium or fresh specimens were used. Since there was variation in pollen size within a species and variation between species was not distinct, pollen size is therefore not a good character for distinguishing the species.

Conclusions

Only in *D. testudinarum* are pollen characters species-specific (Table 3) and the pollen of this species can clearly be distinguished by a combination of its large size, thick wall and verrucose exine. Although there are differences in pollen morphological characters between the species, intraspecific variation also occurs. A combination of pollen characters, such as the exine sculpture, size and shape, can, in some cases (Table 3), provide supplementary information, which should, however, be used together with other characters for the identification of *Durio* species.

Table 3. Diagnostic Pollen Characters of Some *Durio* Species

Characters Species	Exine sculpture	Pollen shape	Polar diameter µm	Equatorial diameter µm
D. griffithii	psilate perforate	oblate spheroidal	(46—)53.5(—60)	(52—)58.5(—62)
D. lanceolatus	scabrate	subprolate	(66—)70.8(—76)	(50—)68.7(—80)
D. oxleyanus	scabrate	oblate spheroidal	(52—)55.1(—56)	(52—)53.7(—56)
D. singaporensis	scabrate	oblate spheroidal	(80—)94(—104)	(80—)81.5(—84)
D. testudinarum	verrucate	oblate spheroidal	(92—)109(—145)	(68—)81.6(—92)

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