Pertanika J. Trop. Agric. Sci. 22(1): 1 - 7 (1999)

ISSN: 1511-3701
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Palynological Study of Bornean *Nepenthes* (Nepenthaceae)

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Keywords: Palynological, pollen, Bornean Nepenthes species, homogeneous

ABSTRAK

Kajian palinologi 27 spesies Nepenthes dari Borneo menunjukkan min tetrad debunga adalah berjulat antara 27 μm hingga 38.9 μm. Julat saiz tersebut adalah terangkum dalam kategori kelas debunga bersaiz kecil dan sederhana. Jenis liang, bentuk permukaan eksin, dan unit debunga dan bentuk adalah seragam bagi semua spesies, dan dengan itu semua sifat tersebut tidak berguna dalam pensempadan spesies dan seksyen. Kajian min saiz debunga mendapati, spesies yang dikaji boleh dibahagi kepada 3 kumpulan iaitu Kumpulan I:27 μm, Kumpulan II:28.5-34.7 μm, dan Kumpulan III:37.2-38.9 μm.

ABSTRACT

Palynological study shows that the mean pollen tetrad size of 27 Bornean Nepenthes species ranges from 27μm to 38.9 μm, thus falling into the small and medium-sized grain class. Aperture type, exine sculpturing, pollen unit and shape are homogeneous among Bornean species and thus not taxonomically useful at the specific and sectional level. The mean pollen tetrad size revealed that the species under investigation can be divided into three groups: Group I: 27 μm, Group II:28.5-34.7 μm, and Group III:37.2-38.9 μm.

INTRODUCTION

Within the last four decades the study of pollen has been expanded to such an extent that it now has an extensive separate literature and is virtually a discipline of its own. Pollen grains can easily be observed and studied under the light microscope but for the study of its finest detail such as wall architecture and exine sculpturing Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM) are necessary. Pollen grains are comparatively little altered by the process of preparing herbarium specimens; thus herbaria have been extensively used as a source of reference material for comparative studies in palynology (Brenan 1968). The fact that fossil pollen retains most of its structural detail is of great significance in establishing evolutionary trends in Angiosperms (Mueller 1970). Among the bases of angiosperm phylogeny, palynology is unique, in that through no other study can one obtain as great an amount of information from so little material in such a short time (Walker & Doyle 1975). An excellent review of the systematic applications of palynology in the plant kingdom has been given by Erdtman (1963) and examples of the usefulness of the pollen characters in delimiting plant taxa are given by Cerceau-Larrival (1971) in the Umbelliferae, Skvarla and Turner (1966) in the Compositae; Erdtman and Metcalfe (1963) gave an example in which palynology and anatomy can be used to solve a taxonomic problem; and Jeffrey (1964) used pollen morphology to establish a new system of classification in the Curcubitaceae.

Features of pollen and spore are being increasingly used in systematic studies including size and shape (the principle characters of pollen grains), pollen type, number and position of the apertures and pollen wall architecture (Heywood 1976).

No palynological study of Bornean *Nepenthes* directly related to taxonomic work has previously been carried out. Som (1988) mentioned an early investigation by Stern (1917), and studies of Kuhl (1933), Lim and Prakash (1973) and Kaul (1982) but each study involved only one, two or three species only. These species includes *Nepenthes melamphora, N. ampullaria, N. gracilis, N. alata* (Philippines), *N. mirabilis* (Ambiona), *N. vieillardii* (New Caledonia); Kaul worked on *N. villosa* and *N. lowii* from Mt. Kinabalu in Borneo. Som (1988) surveyed eight of the eleven species occurring in the Malay Peninsular excluding *Nepenthes* from Singapore Island. Som produced similar findings to the previous workers, that the structure of the grains is generally uniform between species, thus taxonomically significant only at the generic level. Pollen is released in tetrahedral tetrads, the spine is echinate and furrows and apertures are obscured (Som 1988). However, she found that pollen tetrad size differed inter- and intraspecifically and variation in exine sculpturing and including spine shapes, size and density which differed interspecifically.

The two main objectives of this study are firstly to determine the variation of pollen morphological characters within and between the species under investigation and secondly, to determine whether these pollen characters are significant in delimiting species or section of *Nepenthes*.

MATERIALS AND METHODS

The pollen grains study of 27 Bornean *Nepenthes* species were taken from dried herbarium specimens and flowers pickled in kew spirit (50% ethyl alcohol: formalin: glycerol: 18:1:1) and FAA (50% ethyl alcohol: glacial acetic acid: formalin; 18:1:1).

The pollen samples for SEM study were acetolysed following treatment of Walker and Doyle (1975). The pollen was placed in corked centrifuge tubes in acetolysis fluid of acetic anhydride and concentrated sulphuric acid (9:1) in an oven at 50°C overnight. It was then washed once with glacial acetic acid and three times with distilled water.

For pollen tetrad measurement, grains were mounted in glycerine jelly and the slides ringed with nail varnish. Pollen for examination under SEM was air dried on filter paper, then transferred onto double-sided adhesive tape, and coated with gold.

RESULTS

The results of the palynological study are summarised in Table 1, and selectively illustrated in Plates 1-2. Description of the pollen features reported is aperture type, exine sculpturing, pollen unit, shape and size.

Aperture Type and Exine Sculpturing

Apertures are specially delimited, generally thin-walled areas in the outer pollen wall or exine through which the pollen tube usually (but not always) emerges at the time of germination (Walker and Doyle 1975). The pollen grains of all species investigated were lacking a germination furrow or aperture. The pollen grain ornamentation of all the species studied was echinate (Plates 1-2).

Pollen Unit and Shape

All 11 species investigated produce pollen in tetrahedral tetrads, which represent a retention of the four products of meiosis from a single pollen mother cell. Since all the pollen unit of all species is in tetrads, it is not possible to identify the shape of each pollen grain.

Pollen Tetrad Size

The pollen tetrad diameter of the species falls into the medium-sized class for pollen, ranging from 27 µm to 38.9 µm (Table 1). The coefficient of variation calculated for each of these species is low, ranging from 3.8% to 11.1%, standard error values range from 0.2 to 0.6 were calculated with degree of freedom of 119 to 779 and 95% confidence limits for mean range from 0.2 to 0.6 µm.

The species can be divided into three groups. Group I comprises a single species, *N. gracilis* (Section Vulgatae), which has the smallest mean pollen tetrad diameter (27 µm). Group II is represented by species from five sections of Danser (1928). The mean pollen tetrad diameter of the group ranges from 28.5 µm to 34.7 µm and shows continuous variation between all the species. Group III is represented by three species

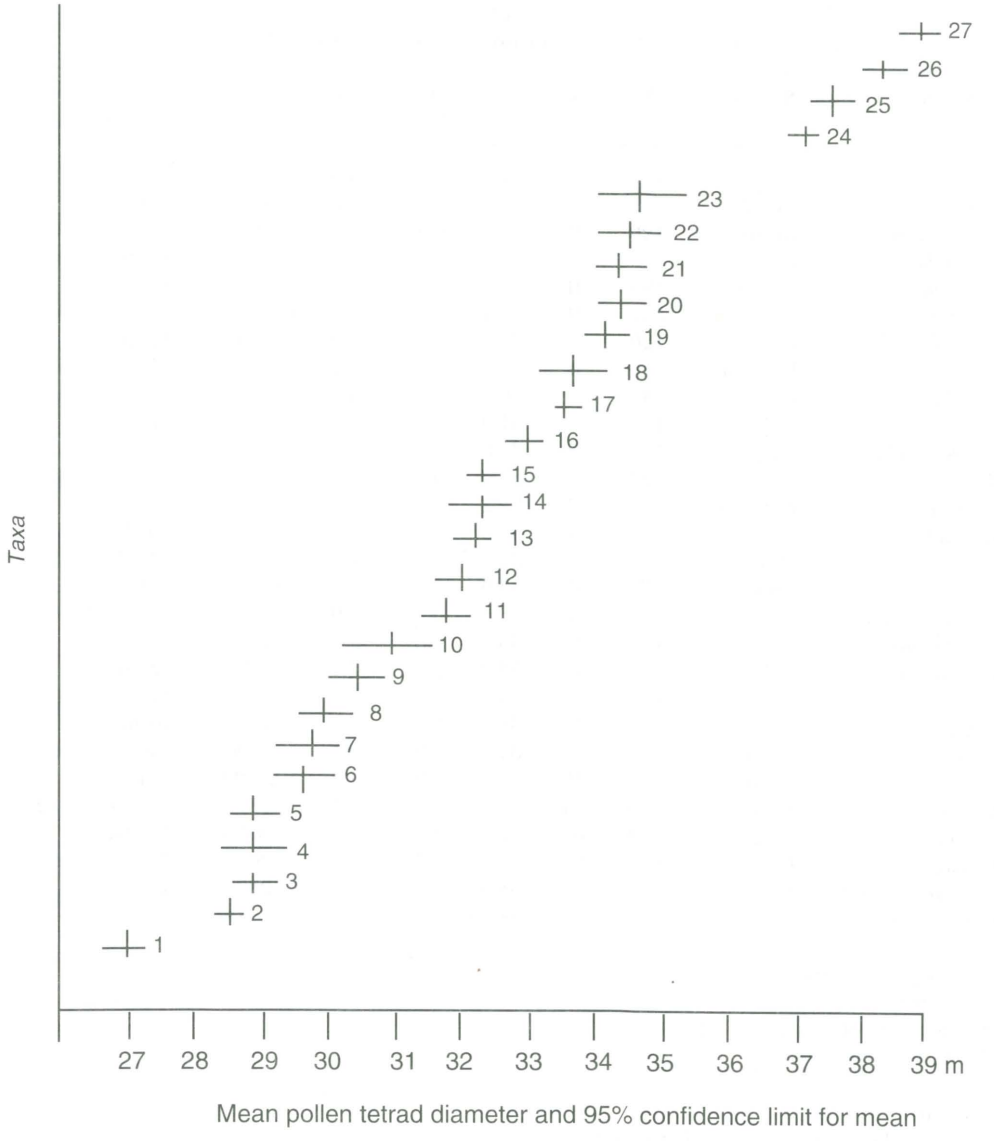

Fig 1. Mean pollen tetrad diameter and 95% confidence limit for mean of 27 Bornean Nepenthes species

KEY

Key to taxa	:	Refer to Table 1
Group I	:	Taxa 1
Group II	:	Taxa 2-23
Group III	:	Taxa 24-27

TABLE 1
Mean pollen tetrads diamaeter of twenty-seven species of Bornean *Nepenthes*

	Species	Section	N	GP	μ	SE	CV	Altitude (m)	Specimens examined
1	*N.gracilis*	*Vulgatae*	120	I	27.0	0.3	6.6	30-80	JI033 & 868
2	*N. ampullaria*	*Urceolate*	495	II	28.5	0.3	9.9	10-200	J2418, 2336, 865
3	*N. bicalcarata*	*Urceolate*	120	II	28.9	0.4	7.5	NR	Fosberg 43860
4	*N.mapuluensis*	*Regiae*	120	II	28.9	0.5	9.2	800	Kostermans 14017
5	*N.hirsuta*	*Nobiles*	120	II	28.9	0.4	7.9	NR	S24068
6	*N.curtisii*	*Regiae*	360	II	39.7	0.3	11.1	1000-1400	J2414 & 950
7	*N. northiana*	*Insignes*	120	II	29.8	0.4	6.0	30	J2378
8	*N.tentaculata*	*Vulgatae*	210	II	29.8	0.4	9.4	1700	Mjoberg 49
9	*N. rafflesiana*	*Insignes*	345	II	30.5	0.4	10.9	0-60	SAN27721
10	*N.mirabilis*	*Vulgatae*	120	II	31.0	0.6	9.8		J1120
11	*N. albomarginata*	*Vulgatae*	120	II	31.8	0.4	6.2	0-30	J2417
12	*N. muluensis*	*Vulgatae*	120	II	32.0	0.4	8.7		J2401 & 2405
13	*N. hookeriana*	*Urceolata*	120	II	32.2	0.3	7.6	150	J2480
14	*N. faizaliana*	*Regiae*	120	II	32.3	0.4	7.6	NR	S441673
15	*N.veitchii*	*Regiae*	390	II	32.3	0.2	7.2	NR	J2391, SAN82496
16	*N. lowii*	*Regiae*	570	II	33.0	0.2	7.8	1700-2000	J2406, 2395, SAN 23341
17	*N. reinwardtiana*	*Vulgatae*	780	II	33.6	0.2	7.0	520-1400	J2481, 2468, 2433, 2429
18	*N.x alisaputraiana*	*Hybrid*	110	II	33.7	0.6	9.0	1900	J2442
19	*N. macrovulgaris*	*Nobiles*	120	II	34.2	0.4	7.1	520	J2467
20	*N. clipeata*	*Regiae*	120	II	34.4	0.4	6.6	NR	Hallier 2344
21	*N. edwardsiana*	*Insignes*	100	II	34.4	0.5	7.7	2600	Sands 3651
22	*N.fusca*	*Regaie*	120	II	34.8	0.6	9.1	1500	Endert 3955
23	*N. rajah*	*Regaie*	300	II	34.7	0.3	7.0	1930-2320	J2443
24	*N. mollis*	*Regiae*	120	III	37.2	0.4	6.1	1800	Endert 4282
25	*N.villosa*	*Insignes*	490	III	37.2	0.2	6.7	1800-3400	J1124, 1190
26	*N. ephippiata*	*Regiae*	120	III	38.5	0.3	3.8	2000	Nootebome 4617
27	*N. kinabaluensis*	*Regiae*	120	III	38.9	0.4	5.1	2800	J2423, 2313-15

N no. of pollen sample
SE Standard error
CV Coefficient of variation (%)
μ Mean diameter(μm)
GP Group
J Jumaat
SAR Sarawak Forest Department Herbarium
SAN Sabah Forest Department Herbarium

of Section Regiae and one species of Section Insignes.

DISCUSSION

Angiosperm pollen grains exhibit a tremendous size range, from 2 μm to 2000 μm (Walker and Doyle 1975). They suggested the primitive size of angiosperm pollen falls largely between 50-99 μm, but pollen size is undoubtedly an easily reversible character and determination of the primitive size class of pollen of any particular taxon must be based on the correlation of pollen size with other characters of the taxon. Som (1988) recorded the pollen tetrad size range of eight Peninsular Malaysian species as from 21 μm 38.4 μm, and pollen grain size from 10 μm to 25 μm. Adam (1998) reported *Nepenthes* species from Borneo have spinose pollen tetrads with diameters ranging from 20 to 40 μm; and according to him the spines may help the pollen to stick to the hairy bodies of the pollinators. This study shows that the mean pollen tetrad size ranges from 27 μm to 38.9 μm, thus falling into small and medium-sized grain class of Walker and Doyle. Thus the genus *Nepenthes* therefore belongs to a derived angiosperm group.

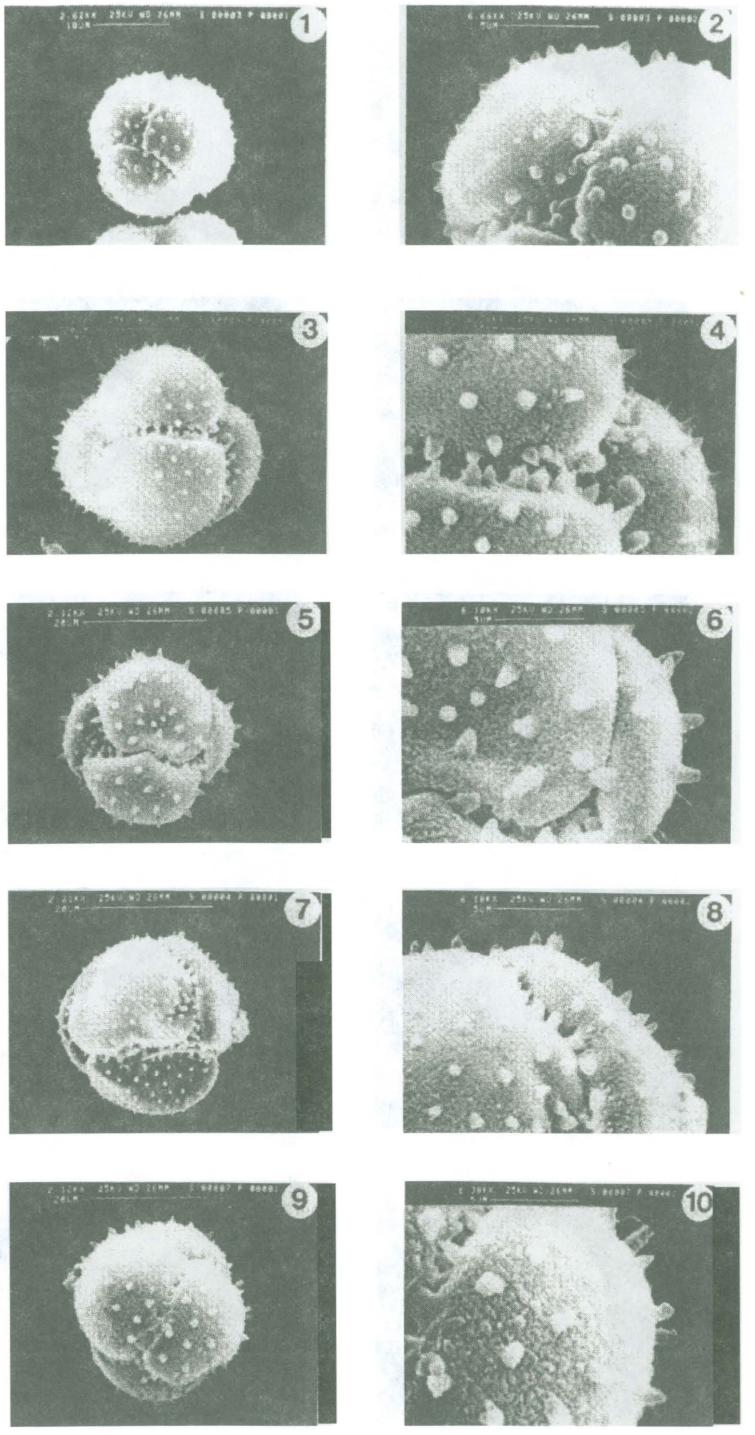

Plate 1. SEM photographs of the pollen tetrads of Bornean *Nepenthes* species

1 *N. mirabilis* 10µm
2 *N. mirabilis* 5 µm
3 *N. reinwardtiana* 20µm
4 *N.reinwardtiana* 5 µm
5 *N. rafflesiana* 20 µm
6 *N. rafflesiana* 5 µm
7 *N. northiana* 20 µm
8 *N. northiana* 5 µm
9 *N.villosa* 20 µm
10 *N. villosa* 5 µm

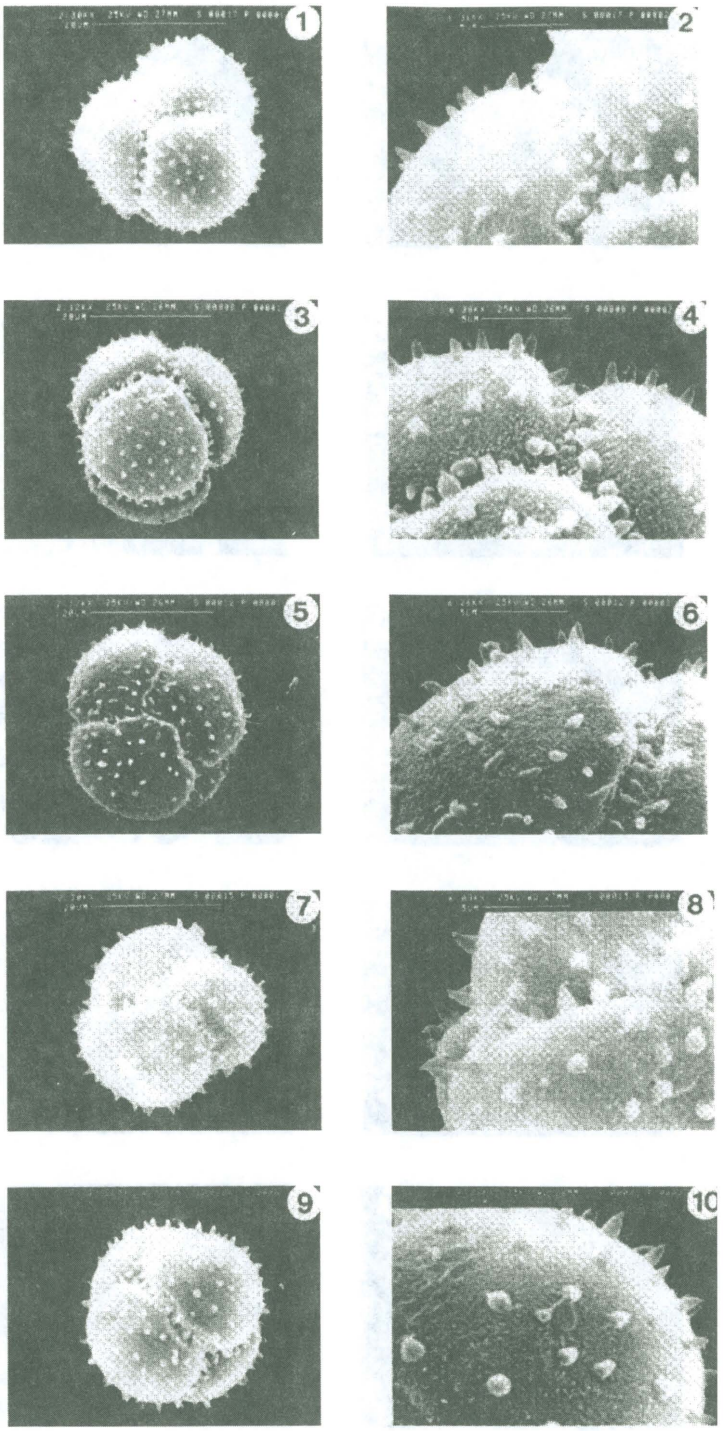

Plate 2. SEM photographs of the pollen tetrads of Bornean *Nepenthes* species

1 *N. veitchii* 10µm
2 *N. veitchii* 5 µm
3 *N. lowii* 20µm
4 *N. lowii* 5 µm
5 *N. mollis* 20 µm
6 *N. mollis* 5 µm
7 *N. rajah* 20 µm
8 *N. rajah* 5 µm
9 *N.x alisaputraiana* 20 µm
10 *N. x alisaputraiana* 5 µm

Danser (1928) suggested that Section Vulgatae was amongst the primitive group while Danser regarded Regiae, with most of its members restricted in distribution and endemic to Borneo, as a younger group, and *N. rafflesiana* (Group II) with the widest distribution range as one of the oldest species in Section Insignes.

This study shows that some members from section Vulgatae have small pollen tetrads whereas most members of section Regiae have the biggest pollen tetrads.

It was reported that pollen grains size might be correlated with elevation (Mueller 1979 in Kiew 1984). In this study most of the high altitude species, particularly members of section Regiae have larger pollen tetrads and lowland species tend to have smaller pollen tetrads.

Numerous reports in the literature suggest that pollen grain size can be a rather unstable character (Som 1988; Kiew 1984; Walker and Doyle 1975; Stanley and Linskens 1974); Walker and Doyle stated that pollen size might be somewhat affected or influenced by method of preparation. Even though chemical treatment and mounting media can influence pollen grain size, these two factors can be eliminated since all the materials in this study were identically treated chemically and mounted in the same media (Stanley and Linskens 1974). Stanley and Linskens (1974) also discussed possible sources of size variation and reported cases where pollen size was correlated with external factors such as mineral conditions, water conditions and temperature and internal factors such as chromosome number and flower character. Further research into these areas may prove useful to determine whether there is any correlation of pollen tetrad size of *Nepenthes* with any of these factors. Kiew (1984) shows that the size of pollen grains in heterostylous Malesian Oleaceae was associated with style length.

Aperture type, exine sculpturing, pollen unit and shape are homogeneous among Bornean *Nepenthes* and thus not taxonomically useful at the specific level and sectional.

ACKNOWLEDGMENTS

We wish to thank Universiti Kebangsaan Malaysia, University of Aberdeen, Malaysian Government Research & Development grant 4-07-03-042, 4-07-03-054, Tabung Biodiversiti Fakulti Sains Sumber Alam and Sabah Parks for financing this project. We are grateful to Director of Sabah Park and Sarawak Forest Department (National Parks Section) for allowing us to collect the specimens for this study; Universiti Kebangsaan Malaysia for granting the sabbatical leave to the first author (JHA); Curator Herbarium Universiti Kebangsaan Malaysia (UKMS), Herbarium Sarawak Forest Department (SAR), Herbarium Sabah Forest Department (SAR), Herbarium Bogoriense (BO); Sabah Park Herbarium; Herbarium Kew (K), Rijksherbarium Leiden (L) for the herbarium specimens; Mr. Kevin Mackenzie for his technical assistance with scanning electron microscope and Mr. Norman Little for the SEM photographs; Dr. Ramlan Omar for spending his time editing the SEM photographs; Mr. Julaihi Haji Adam and Mr. Aliosman Mahdi for the assistance in the field; and Mrs Aspah Hashim for typing this manuscript.

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(Received 30 April 1998)
(Accepted 20 April 1999)