ISSN 0215 - 4706

Floribunda

Floribunda 3(3): 57–84. April 2007

DAFTAR ISI

Terakreditasi B berdasarkan Keputusan Dirjen Dikti Depdiknas RI No. 26/DIKTI/Kep./2005

Dewan Penyunting *Floribunda* amat berterima kasih kepada
Prof. Dr. Edi Guhardja (IPB Bogor)
Prof. Dr. Mien A. Rifai (AIPI Jakarta)
Prof. Dr. Ryoko Imaichi (Japan)
Prof. Dr. Eko Baroto Walujo (LIPI Bogor)
Prof. Dr. Wasmen Manalu (IPB Bogor)
Dr. Irawati (LIPI Bogor)
Dr. Mami Konomi (Japan)
Dr. Mamiko Sato (Japan)
Dr. Chie Tsutsumi (Japan)

atas kesudiannya bertindak selaku mitra bestari untuk terbitan
Floribunda 3(3)

Terbit: April 2007 Tanggal terlaksana: 1 Desember 2009

***

Penggalang Taksonomi Tumbuhan Indonesia ingin merekamkan rasa terima kasihnya pada Program Hibah Simposium Nasional Organisasi Profesi Direktorat Penelitian dan Pengabdian Kepada Masyarakat - DIKTI untuk ikut mendanai penerbitan *Floribunda* 3(3)

ISSN 0215 – 4706

Floribunda

Floribunda merupakan organ resmi Penggalang Taksonomi Tumbuhan Indonesia, diterbitkan dua kali setahun dan menerbitkan makalah dalam bahasa Indonesia dan Inggris mengenai pelbagai gatra sistematika keanekaragaman flora Malesia pada umumnya dan Indonesia pada khususnya yang berasal dari hasil penelitian, pengamatan lapangan, pengalaman pribadi, telaahan bergagasan, dan tinjauan kritis.

Sidang Penyunting

Ketua Penyunting
Tutie Djarwaningsih (BO)

Penyunting
Tri Mulyaningsih (UNRAM)
Atik Retnowati (BO)
Novita Kartika Indah (UNESA)
Titien N. Praptosuwiryo (KRI)
Nunik Sri Ariyanti (IPB)

Penyunting Pelaksana
Himmah Rustiami (BO)

Tata Letak
Muhamad Ruslan (BO)

Petunjuk kepada pengarang

Jenis tulisan

Makalah lengkap memuat hasil penelitian floristik, revisi, atau monografi unsur-unsur flora Malesia.

Komunikasi pendek mencakup laporan kemajuan kegiatan penelitian, pengembangan dan rekayasa keanekaragaman flora Malesia yang perlu segera dikomunikasikan.

Tulisan lain meliputi obituari tokoh keanekaragaman flora, tinjauan kritis bergagasan, telaahan serta pembahasan persoalan aktual seputar kegiatan penelitian, pengembangan dan rekayasa tetumbuhan Indonesia, serta timbangan buku akan dimuat berdasarkan undangan.

Rujukan pembakuan

Pemakaian Bahasa Indonesia sepenuhnya mengikuti *Pedoman Umum Ejaan yang Disempurnakan, Pedoman Umum Pembentukan Istilah, Kamus Besar Bahasa Indonesia,* serta kamus-kamus istilah yang dikeluarkan Pusat Bahasa. Bahasa Inggris yang dipakai adalah the Queen English dengan berpedoman pada *The Oxford Dictionary of the English Language.* Ketentuan-ketentuan yang dimuat dalam *Pegangan Gaya Penulisan, Penyuntingan,* dan *Penerbitan Karya Ilmiah Indonesia,* serta *Scientific Style and Format: CBE Manuals for Authors, Editors, and Publishers,* dan buku-buku pegangan pembakuan lain akan sangat diperhatikan. Kepatuhan penuh pada *International Code of Botanical Nomenclature* bersifat mutlak.

Gaya penulisan

Penulisan naskah yang akan diajukan supaya disesuaikan dengan gaya penulisan yang terdapat dalam nomor terakhir terbitan *Floribunda.*

Abstrak informatif supaya diberikan dalam bahasa Indonesia dan Inggris yang masing-masing tidak melebihi 200 kata. Sediakan sekitar 7 kata kunci untuk keperluan pengindeksan dan pemindaian.

Bilamana diperlukan ucapan terima kasih dan bentuk persantunan lain dapat dicantumkan sesudah tubuh teks tetapi sebelum daftar pustaka.

Pengacuan pada pustaka hendaklah dilakukan dengan sistem nama-tahun. Daftar pustaka supaya disusun berdasarkan alfabet nama pengarang dengan memakai sistem Harvard.

Gambar dan tabel merupakan pendukung teks sehingga perlu disusun secara logis dalam bentuk yang mudah dimengerti. Data supaya disajikan dalam bentuk teks atau tabel atau sebagai gambar, tetapi tidak dalam bentuk ketiganya sekaligus. Siapkan gambar yang lebarnya dua kolom cetak.

Penyumbangan naskah

Naskah dikirimkan dalam bentuk ketikan atau cetakan komputer pada kertas HVS berukuran A4 bersama-sama dengan disket komputer yang diprogram untuk serasi dengan IBM, atau melalui *e-mail.*

Naskah yang ingin diterbitkan dalam *Floribunda* akan dipertimbangkan pemuatannya *hanya* jika pengirimannya disertai pernyataan tertulis dari 2 (dua) orang mitra bestari yang dipilih sendiri oleh penulisnya (akan lebih diutamakan bila mitra bestari dipilihkan dari luar lingkungan kerja penulis), yang menyatakan bahwa secara ilmiah keorisinalan dan makna sumbangan naskah tersebut memang layak diterbitkan.

Pengolahan naskah

Sidang penyunting bersama sekelompok mitra bestari akan mengaji ulang kesesuaian isi dan keselarasan format setiap naskah dengan *Floribunda.* Perubahan yang dilakukan akan dikomunikasikan kepada penulis dalam bentuk contoh cetak akhir sebelum diterbitkan.

Cetak lepas

Penulis menerima 5 cetak lepas dari tulisannya secara cuma-cuma.

Kantor penyunting

Sidang Penyunting *Floribunda*
Herbarium Bogoriense, Cibinong Science Center
Jalan Raya Bogor KM 46 Cibinong 16911
Telepon : (021) 8765066-67
Fax : (021) 8765059
E-mail : herbogor@indo.net.id

SPECIFIC DELIMITATION AND RELATIONSHIP AMONG SPECIES OF DIPLAZIUM BASED ON SPORE MORPHOLOGY

Titien Ngatinem Praptosuwiryo
Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, IPB, Darmaga, Indonesia
(Permanent address: Center for Plant Conservation – Bogor Botanic Gardens
Indonesian Institute of Sciences. Jl. Ir. H. Juanda No. 13 Bogor, Indonesia)
Masahiro Kato
Department of Botany, National Museum of Nature and Science, 4–1–1 Amakubo Tsukuba 305-0005, Japan
Dedy Darnaedi
Herbarium Bogoriense, Pusat Penelitian Biologi – LIPI, Cibinong Science Center, Indonesia

Titien Ngatinem Praptosuwiryo, Masahiro Kato & Dedy Darnaedi. 2007. Pembatasan Jenis dan Hubungan Kekerabatan diantara Jenis *Diplazium* Berdasarkan Morfologi Spora. *Floribunda* 3(3): 57–76. — Morfologi spora 26 jenis *Diplazium* dari Malesia Barat telah diamati dengan menggunakan mikroskop cahaya (LM) dan mikroskop elektron payar (SEM). Semua jenis memiliki spora monolet, simetri bilateral, heteropolar, bentuk garis besar polar elip, berperin dengan *laesura* ditutupi perin. Berdasarkan gambar SEM dari morfologi spora, dikenali sembilan group utama yaitu: 1). Spora yang memunggung bukit, tidak berjendelaan dan dihiasi dengan pola-pola mirip sayap menonjol membentuk punggung bukit tumpul (Group I, misalnya *D. bantamense*); 2). Spora dengan memunggung bukit-bersayap, berjendelaan, lubang-lubang mikroskopis tersebar secara tidak teratur (Group II, misalnya *D. subserratum*); 3). Spora dengan perin bersayap dan berjendelaan, lubang-lubang mikroskopis tidak teratur (Group III, misalnya *D. crenatoserratum*); 4). Spora beralur karang dan berjendelaan, dengan jendela-jendela tidak teratur atau lubang-lubang besar dan dihiasi dengan dabus lebat tidak teratur pada pinggir muri seperti sayap (Group IV, misalnya *D. silvaticum*); 5). Spora berjendolan mikroskopis tanpa muri seperti sayap atau punggung bukit (Group V, misalnya *D. esculentum*); 6). Spora dengan hiasan perin bersayap tidak berjendelaan (Group VI, misalnya *D. tomentosum*); 7). Spora yang berperin berdabus dan tidak berjendelaan (Group VII, misalnya *Diplazium* sp.1); 8). Spora yang mempunyai bentuk punggung bukit-bersayap, beralur karang dan berdabus (Group VIII, misalnya *D. spiniferum*); and 9) spora yang bersayap, berdabus dan berjendelaan (Group IX, misalnya *Diplazium* sp.2). Morphologi spora memperlihatkan bahwa hiasan perin mendukung pembatasan jenis *Diplazium.* Oleh karena itu kunci identifikasi jenis berdasarkan morfologi spora disajikan. Walaupun demikian analisa filogeni dengan menggunakan parsimoni memperlihatkan bahwa variasi morfologi spora tidak cukup untuk menggambarkan hubungan kekerabatan alami diantara jenis *Diplazium*.

Kata kunci: Malesia Barat, *Diplazium,* morfologi spora, pembatasan jenis, hubungan kekerabatan, filogenetik.

Titien Ngatinem Praptosuwiryo, Masahiro Kato & Dedy Darnaedi. 2007. Specific Delimitation and Relationship among Species of *Diplazium* Based on Spore Morphology. *Floribunda* 3(3): 57–76. — The spore morphology of 26 species of *Diplazium* from West Malesia were examined under LM and SEM. All species have monolete, bilateraly symmetrical, heteropolar, polar outline elliptical, perinates with laesura concealed by its perine. Nine main groups of spores morphology are recognized based on spore morphologies: 1). Spores that are costate, non-fenestrate, and ornamented with a wing-like pattern raised to form a pattern of slightly rounded ridges (Group I, e.g. *D. bantamense*); 2). Spores with costate-alate, fenestrate, micro holes irregularly scattered (Group II, e.g. *D. subserratum*); 3). Spores that have alate and fenestrate perine, irregular micro holes (Group III, e.g. *D. crenatoserratum*); 4). Coralline and fenestrate spores, with irregular windows or large holes and decorated with irregular dense echinae on the terminating margins of wing-like muri. (Group IV, e.g. *D. silvaticum*); 5). Micro rugulate spores without wing-like muri or costae (Group V, e.g. *D. esculentum*); 6). Spores with alate non-fenestrate perine sculpture (Group VI, e.g. *D. tomentosum*); 7) Spores that echinate perine and non-fenestrate (Group VII, *Diplazium* sp.1); 8). Spores that have form costate-alate, coralline and echinate (Group VIII, e.g. *D. spiniferum*); and 9). Spores form that are alate, echinate and fenestrate (Group IX, e.g. *Diplazium* sp.2). Spore morphology study showed that perine ornamentations support in delimitating

species of *Diplazium*. Therefore a key for species based on spores morphological characters is presented. However, the phylogenetic analysis using parsimony revealed that morphological variation of spore is inadequate to depict natural relationship among *Diplazium* species.

Keywords: West Malesia, *Diplazium,* spore morphology, specific delimitation, phylogenetic relationship.

Diplazium is a terrestrial ferns which is commonly found in the moist ground at the humus, both in the primary and secondary forest at 20–2500 above sea level. It is the largest genus in *Dryopteridaceae* sensu Kramer et al. (1990) and generally distinguished from closely related genera, for example *Athyrium* (Kato 1977) by a combination of several characters, such as the brown to blackish scales, vascular bundle U- or V-shaped, frond architecture, and linear double sori.

Diplazium consists of about 400 species, distributed mainly in the tropics and sparingly in temperate forest (Copeland 1947, Kramer et al. 1990). It is predicted that 75% of species in the world (ca. 300 species) occur in Malesia (Roos 1995).

Diplazium is very difficult and quite insufficiently known. The young plants may be fertile and difficult to assign to a species (Kramer et al. 1990). Many taxa are considerably morphologically diversified. Many species show continuous morphological variations through apparently intermediate forms, which are commonly regarded as putative hybrids (Takamiya et al. 1999). Consequently specific delimitation in *Diplazium* is very difficult.

Ferns spores display remarkable variations and have been extremely helpful in systematic studies. In many cases, particular spore morphology is distinctive for families or genera, or in some instance individual species. Extensive studies on the spore of Pteridophyta showed that spore morphology can be used as one of the taxonomic evidence for delimiting taxa. Ferrarini et al. (1986) reported that a number of taxa based on spore illustration by SEM of the native Pteridophyta in Italy is 124 specific and intraspecific taxa. They showed that spore morphology provides evidence to support taxonomic work for delimiting, both generic and species level in the Pteridophyta. Even they recognized some infraspecific of *Asplenium* based on its spore morphological characters, such. *A. trichomanes*, *A. ruta-muraria*, *A. seelosii* and *A. officinarum*. Harris (1955) described spores of 170 taxa of New Zealand fern based on herbarium specimens. Forty six years after Large & Braggins (1991) revised this work and provided full spores descriptions of 211 species from New Zealand based on samples taken from fresh material. These works also revealed that spore morphology among the genera in a family are diversified so that a tentative key to genera of pteridophytes from New Zealand is given. However, they did not discuss the correlation between palynological characters and its taxomical treatments. Ohta & Takamiya (1999) used spore morphology characters that observed by using SEM to distinguish *Diplazium griffithii* from other species in the *Diplazium mettenianum* complex.

Palynological characters may also provide evidence to illustrate the pattern of relationship. Blackmore (2000) showed the use of pollen morphology, in isolation, as a means of explicitly illustrating the relationship pattern of species included in subtribe *Scorzonerinae* (*Asteraceae*). The direction of the transformation was determined by method of outgroup comparison, because it is very difficult to determine the transformation of characters on the basis of the evolutionary trend approach. Even it is difficult to polarize characters on the basis ontogeny. Therefore one solution to the issue of character polarization is to analyze the characters as unordered, or unpolarized, unless evidence development for the transformation is available, and it allows parsimony to determine the direction of change.

The exospore is considered to be extremely important for establishing evolutionary relationship (van Konijnenberg-van Citteret 1999). Exospore architecture is very diverse. The detailed exospore structure revealed by light and electron microscopy was first studied by Pettit (1966). Since then, many pteridologists have dealt with the subject and its culminated with the work of Tryon and Lugardon (1991) which describes pteridophytes spores from all over the world using SEM and TEM (Transmission Electron Microscope).

Any determination of what constitutes a primitive (ancestral) or derived state in the structure of fern spores must be based on homologies. Connecting this subject, Van Konijnenburg-van Cittert (1999) discussed the evolution of various characters of spore shape and wall structure based on fossil evidence. The characters discussed include number of spores per sporangium, spore size, overall spore shape in connection with laesura, thickness and sculpture of exospore, absence or presence of a perispore, and its sculpture. The evolutionary trend

in fossil fern spores are summarized as follow: (1). A number of 256 spores per sporangium is primitive, while 128 or less spores per sporangium is derived. There is a trend of reduction of the number of spores per sporangium; (2). Standard of spore diameter is between 30 and 60 µm. Spore diameter over 60 µm is derived. Spore diameter under 30 µm may be derived, but it is probably the most primitive stage; (3). Tetrahedral, trilete spores are primitive. Bilateral, monolete spores are derived. The evolution from trilete to monolete spores are seen in several fern families. Trilete and monolete spores may even occur in one sporangium; intermediates have been recorded; (4). The most primitive overall spore shape in trilete spores is probably globose spores, while (rounded) triangular spores are derived; (5). Exospores between 1 and 2 µm are usually primitive, but thin and smooth exospores correlated with a thick and elaborate parispore may be derived. Exospores over 3 µm and certainly over 6 µm, are derived; (6). Smooth or fine exospore ornamentation is possibly primitive, but in the *Schizaeceae* the oldest recorded spores appear only late during evolution; (7). Exospores sculptures that are certainly derived are: bisculpate spores; strongly raised laesurae; laesurae bordered by margo; valvate spores; and elaboration of the equatorial region; (8). Absence of perispore is primitive; presence of perispore is derived; and (9). Thin, smooth or finely ornamented perispores consisting usually of more than one layer and having an elaborate ornamentation are derived.

This paper present spore morphology of *Diplazium* from West Malesia observed by using both LM and SEM. Due to the availability of spores collection, only 26 species were observed. The aims of the research were: (1). To get supporting data for species delimitation on *Diplazium*; (2). To provide a tentative key for species based on spore morphology; and (3). To infer phylogenetic relationship between species in the genus *Diplazium* based on spore characters.

MATERIALS AND METHODS

Spores of twenty six species of 46 collection numbers of *Diplazium* were observed by using SEM for getting detail of its perine ornamentation. The list of collection number examined are presented on Table 1. These species were chosen based the spore sample availability.

Spores sample were collected both from fresh material and herbarium material housed at Herbarium Bogoriense (BO). For determining its size, both polar (P) and equatorial (E) measurements, and morphological outline, 65 collections number were observed by using LM.

a. Preparation for LM

Spores sample, both obtained from fresh spores (air drying without alcoholic treatments) and from herbarium specimens, were directly transfered on slides and mounted with glycerine jelly for study with light microscopy. Spore size, polar (P) and equatorial (E) measurements, were taken for each spores. Twenty spores from each individual plant was taken randomly. Each species was represented by one to five collection numbers.

b. Preparation for SEM

Separation and Attaching Spores on the Holder. Spores sample used were fresh spores (air drying without alcoholic treatments) and spores of herbarium specimens housed at BO. Spore samples were separated from the indusium and sporangium fragment of indusium. Separation was conducted manually. One spatula of spora sample were spread over on waxpaper 4.5 x 4.5 cm, then shaked by hand. Spores will separate from the fragments.

Platinum Palladium Coating. Spores were spread over on the holder with carbon tapes. Spores on the holders were sputter-coated with platinum palladium using Ion Sputter (E-102, Hitachi, Japan). After that spores sample were coated with Platinum Carbon by using HITACHI HCP-2 for 10 minutes by using Electron Beam Coating Method.

SEM Observation and Taking Pictures. Spores samples on the holder coated by Platinum Carbon were observed at 5 kV in electron microscope Hitachi S-800. Spores pictures were taken on the longitudinal ekuator, transversal ekuator and polar views.

c. Description format and taxonomic characters

Description are given in full for each taxon and terminology used to describe spores morphology follow those used by Harris (1955) and Large & Braggins (1991).

d. Phylogenetic Analysis

Matric data (Table 3.) was analyzed using maximum parsimony, PAUP Version 4. (Swofford 1998), with the Heuristic search settings. All characters are of type unorder and have equal weight. Gaps are treated as "missing". Starting tree(s) obtained via stepwise addition. *Athyrium filix-femina* was used as reference taxon, out group. Number of trees held at each step during stepwise addition = 1. Branch-swapping algorithm was runned by using tree-bisection-reconnection (TBR).

RESULTS AND DISCUSSION

Spore Characters of *Diplazium* and Its Use in Supporting Species delimitation and Identification

Kramer et al. (1990) described general morphological chraracteristic of *Diplazium* spores, viz. with long, usually prominent, wing-like folds, often with echinate borders, and sometimes cristate or echinate, but without giving further explanation for the existence of the grouping based on spores characters. Roubik (2003) showed alate spores of *D. grandifolium* of Barro Colorado.

All *Diplazium* species from West Malesia have similarities on spores morphological characters as follow: golden yellow to brown when released, monolete, bilaterally symmetrical, and heteropolar; polar outline elliptical, sides convex; equatorial longitudinal view concave-convex to plano convex; equatorial transverse view, proximal face planar to concave, distal face convex to hemispherical. SEM observations revealed that a thick perine is present; laesura is often concealed by perine. Perine is alate to costate alate, wing-like folds. The description of each species is presented on Table 1.

Character used to describe *Diplazium* spores are as follow: 1). Pattern: The general pattern of the perine, appear to be: (a). folded; or b). spinulose; 2). Fenestration: perine; (a). fenestrate throughout; (b). fenestrate in the lacunae of any reticulation; or (c). without fenestrae; 3). Overall ornamentation: (a). smooth; (b). veined or wrinckled; or (c). raised ribs within the lacunae. Large & Braggins (1991) mentioned one more character state, viz. (d) tuberculate with knob-like elements, however this character state is not found for *Diplazium* of Western Part Malesian Region. 4). Pattern density: Density classes are (a). sparse; and (b). dense; 5). Perine fold type: Perine folds may vary, general forms are (a). winged (alate); or (b). ridged (costate); ridges may be rounded or slightly crested; 6). Fold margins: The edges of the perine folds may (a). extend into serrate to echinate projections, (b). bear minute teeth, papillae, or (c). be smooth.

Based on the characters used in describing the spore, the West Malesian *Diplazium* fall into nine main pattern groups based on the perine ornamentation. The nine group are discussed as follows.

(1). Group I. The spore forms most common among the diplazioid ferns of Western Part of Malesian region are costate, non-fenestrate, and ornamented with a wing-like pattern raised to form a pattern of slightly rounded ridges. *D. accedens, D. bantamense, D. lobbianum, D. polypodioides, D. pallidum, D. procumbens, D. sorzogonense, D. speciosum, D. subpolypodioides, D. dilatatum,* and *D. xiphopyllum* are included in this group. The existence of additional ornaments, however, would differentiate among the species. Reticulation type of wing-like muri or wing-like costae, terminating margin of wing-like muri, and surface of perine (both on lacunae and wing-like muri) of these members are in varying character states. Therefore these characters can be used to support species delimitation. Spores of *D. accedens, D. bantamense* and *D. polypodioides* are similar appearance. They are seen incomplete reticulation of wing-like muri or costae often incomplete with terminating margins entire or smooth. *D. accedens* and *D. polypodioides* differ with *D. bantamense* in the projection of wing-like muri or costae. The two first species showed wing-like muri or costae project up to 7 µm, while the third species has wing-like muri or costae project up to 13 µm. *D. accedens* and *D. polypodioides* differ in the size of their irregular polygons lacunae. The first species is with 10 ìm or more across, while the second species 9 ìm or less across.

(2). Group II. The second group (*D. subserratum* and *D. vestitum)* has spores with costate-alate and sparsely fenestrate perine, micro holes are irregularly scattered. The existence and its density of the pappilae on the wing-like muri would be important in differentiating taxa in this group. *D. subbserratum* showed entire terminating margin of wing-like muri, while *D. vestitum* has ciliate wing-like of muri.

3). Group III. The third group (*D. crenatoserratum* and *D. prescottianum)* has alate and sparsely fenestrate perine spores. Additional decoration of perine on both lacunae and wing-like muri add the variation of spore sculpture of this group.

(4). Group IV. Spores of *D. silvaticum* are very distinctive among the diplazioid species of Western Part of Malesian region. Its wings-like muri and lacunae are coralline and fenestrate, irregular windows or holes large (ca. 0.5–1.5 µm across) and decorated with irregular dense echinae on terminating margins of wing-like muri.

(5) Group V. One species of *Diplazium* showed a rather simple ornamentation is *D. esculentum*. Its perine is micro rugulate without wing-like muri or costae. Spores with monolete, bilaterally symmetrical, elliptical, sides convex and regulate perine is also found in other genera, such as

Athyrium. Using LM, Tseng-Chieng (1981) observed spores of this species under the name *Anisogonium esculentum* and described that its perine are reticulate with scabrate processes. As reported by Large & Braggins (1991), spores with micro-rugulate perine is showed in *Athyrium filix-femina*. However, this species is decorated with costae sparsely that formed shallow and very large lacunae. *A.erythropodum* and *A. vidalii* of Thailand observed by Tseng-Chieng (1981) were also revealed rugulate spores.

(6). Group VI. Alate non-fenestrate perine sculpture can be seen in *D. cordifolium*, *D. tomentosum*, *D. simplicivenium*, *Diplazium* sp.3,

Table 1. Spores Description of *Diplazium* in West Malesia.

No.	Species	Spores description & specimen examined
1.	*D. accedens* Blume	Monolete, bilaterally symmetrical, heteropolar; polar outline elliptical, sides convex; equatorial longitudinal view concave-convex to plano-convex; equatorial transverse view, proximal face planar to concave, distal face hemispherical; perinate. E: 25.34(31.47)36.02±2.54; P: 15.32(19.51)23.59±2.82. Laesura: concealed by perine ridge. Perine: costate-alate, reticulation often incomplete; lacunae shallow, project 10–17 µm;wing-like muri or costae project c.0.5–6 µm, terminating margins entire. Exine: often visible through perine, smooth under LM, smooth under SEM. Specimen examined. Java: West Java, Mt. Gede, T.Ng. Praptosuwiryo 1161.
2.	*D. bantamense* Blume	Monolete, bilaterally symmetrical, heteropolar; polar outline elliptical, sides convex; equatorial longitudinal view concave-convex; equatorial transverse view, proximal face concave, distal face hemispherical; perinate. E: 42.81(53.88)66.05±5.69; P: 22.64(31.48)37.47±3.38. Laesura: concealed by perine ridge. Perine: costate-alate, loosely reticulate irregular envelope, costae form a large reticulation; separated from the spores; reticulation often incomplete; lacunae large irregular polygons 11–25 µm across; costae or alate project 3–13 µm, terminating margins entire; surface of perine smooth. Exine: often visible through perine, smooth under LM. Specimens examined. Java: West Java, G. Halimun, T.Ng. Praptosuwiryo 1707, 1766.
3.	*D. cordifolium* Blume	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical, sides convex; equatorial longitudinal view (excluding perine) plano-convex; equatorial transverse view, proximal face planar, distal face convex; perinate. E: 37.02(42.15)51.62 ±5.09; P: 20.23(26.56)31.21±2.97. Laesura: concealed by perine wing. Perine: alate to costate-alate, loose reticulate; irregular envelope separated from exine surrounds the spore in continuous anastomosing wings, forming a loose reticulation; lacunae large irregular polygons 15–20 µm across; thin wing-like muri project 2–8 µm, terminating margins are often echinate; surface of perine sparsely echinate or ciliate; echinae project 0.5–0.8 µm. Exine: visible through perine, smooth under SEM. Specimens examined. Java: West Java, Mt. Salak, T.Ng. Praptosuwiryo 1367, 1457; Mt. Halimun, T.Ng. Praptosuwiryo 1708, 1709, 1775.
4.	*D. crenatoserratum* (Blume) Moore	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical, sides convex; equatorial longitudinal view (excluding perine) concave-convex; equatorial tranverse view, proximal face concave, distal face convex; perinate. E: 29.86(38.19)45.74±4.95; P: 17.92(23.48)27.36±2.94. Laesura: concealed by thin wing-like muri. Perine: alate, loosely reticulate; irregular envelope, separated from exine, surrounds the spore in anastomosing wings, forming loose reticulation, reticulation irregular and often incomplete; lacunae irregular polygons, 8–16 µm across, irregular holes and small papillae within; holes caused by the fallen papillae; muri thin, wing-like, projected c. 1–8 µm, terminating margin ciliate; surface of perine fibrous-like and holed, holes irregular and formed by fallen ciliae. Specimens examined. Sumatra: Jambi, Bukit Dua Belas National Park, T.Ng. Praptosuwiryo 2075. Borneo: East Kalimantan, Bukit Bangkirai, T.Ng. Praptosuwiryo 870, 876.

Table 1. Continued

No.	Species	Spores description & specimen examined
5.	*D. dilatatum* Blume	Monolete, bilaterally symmetrical (made asymmetric by prine), heteropolar, polar outline (excluding perine) elliptical, sides convex; equatorial longitudinal view (excluding perine) concave-convex; equatorial transverse view, proximal face concave, distal face convex; perinate. E: 31.85(45.69)49.56±4.78; P: 19.84(28.55)34.62±3.89. Laesura: concealed by perine. Perine: alate to costate-alate, irregular envelope separated from exine surrounds the spore; wing-like muri projected c.6–15 µm, terminating margin are often ciliate; surface of perine smooth. Exine: visible through perine, smooth under SEM. Specimen examined. Bali: Batukahu Nature Reserve, Bukit Pohen, J.J. Afriastini Bl-170.
6.	*D. esculentum* (Retz.) Swartz	Monolete, bilaterally symmetrical, heteropolar; polar outline elliptical, sides convex; equatorial longitudinal view concave-convex to plano-convex; equatorial transverse view, proximal face planar to concave, distal face hemispherical; perinate. E: 31.72(38.39)43.09±3.35; P: 22.74(26.37)29.12±1.39. Laesura: not visible, concealed by perine. Perine: smooth under LM, micro rugulate under SEM. Exine: often visible through perine, granulate under LM. Specimen examined. Java: East Java, Meru Betiri National Park, T.Ng. Praptosuwiryo 637.
7.	*D. lobbianum* Moore	Monolete, bilaterally symmetrical (made asymmetrical by perine), heteropolar; polar outline elliptical, sides convex; equatorial longitudinal view concave-convex to plano-convex; equatorial transverse view, proximal face planar to concave, distal face hemispherical; perinate. E: 38.20(46.10)51.87±3.22; P: 20.47(26.28)32.81± 3.73. Laesura: concealed by perine ridge or wing-like muri. Perine: costate-alate; wing-like muri surrounds the spore without forming reticulation or with forming loose reticulations, terminating margins ciliate; surface of perine smooth to scatterly ciliate or pappilate. Exine: often visible through perine under LM, smooth under SEM. Specimen examined. Java: West Java, Mt. Gede, T.Ng. Praptosuwiryo 1340.
8.	*D. lomariaceum* (Christ) Price	Monolete, bilaterraly symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) elliptical; equatorial longitudinal view (excluding perine) biconvex; equatorial tranverse view, proximal face convex, distal view hemispherical; perinate. E: 35.03(39.41)54.55±4.20; P: 19.55(24.57)30.13±3.46. Laesura: concealed by wing-like costae. Perine: alate to costate-alate, alae forming loose reticulation; irregular envelope separated from exine surrounds the spore in wing-like muri reticulation, lacunae large, 13–21µm; wing-like muri thin, project 3–10 µm, terminating margin waved or almost entire. Exine: visible through perine, rough rugulate under SEM. Specimen examined. Borneo: South Kalimantan, G. Besar, M. Kato, Gen Murata & JP Mogea B7407.
9.	*D. malaccense* Presl	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) elliptical; equatorial longitudinal view planar convex to concave-convex; equatorial transverse view, proximal face concave, distal view convex. Laesura: concealed by wing-like muri. E: 37.73(34.24)27.53±3.03; P: 26.01(20.80)13.33±2.82. Perine: alate to costate-alate, often loosely reticulate; irregular envelope separated from exine surrounds the spore in continuous anastomosing wings, forming a loose reticulation; wing-like muri project 1.5–8 µm, terminating margins sparsely ciliate. Exine: visible though perine, smooth granulate under SEM. Specimens examined. Borneo: East Kalimantan, Bukit Bangkirai, T.Ng. Praptosuwiryo 831, 9443.
10.	*Diplazium* sp. 3	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical, sides convex; equatorial longitudinal view (excluding perine) concave-convex; equatorial transverse view, proximal face convex, distal face convex; perinate. E: 39.61(51.42)57.01±4.28; P: 18.51(31.49)35.85±3.59. Laesura: concealed by perine. Perine: alate, alate sometimes loosely reticulate; wing-like muri project 4–13 µm, terminating margins almost entire. Specimen examined. Java: West Java, Mt. Salak, T.Ng. Praptosuwiryo 1451.

Table 1. Continued

No.	Species	Spores description & specimen examined
11.	*D. pallidum* (Blume) Moore	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical; equatorial longitudinal view (excluding perine) plano-convex; equatorial view tranverse view proximal face convex, distal face hemispherical; perinate. Size: E: 32.65(47.96)65.02±8.98; P: 17.08(27.09)34.12±5.12. Laesura: concealed by perine. Perine: alate under LM, costate under SEM, irregular envelope separated from exine surrounds the spore in irregular, wing-like costae, often anastomosing to form loose reticulation, lacunae shallow irregular polygons 15–18 µm wide, muri 0.8–5 µm wide, surface of muri smooth and lacunae smoothly granulate. Exine: visible through perine, granulate under LM. Specimens examined. Malay Peninsula: Pahang, S. Burong, Holttum SFN 31350. Sumatra: West Sumatra, Mt. Talang, J. v. Borssum W. 2787. Java: East Java, Mt. Wilis, T.Ng. Praptosuwiyo 1688; West Java, Mt. Halimun, T.Ng. Praptosuwiryo 1759.
12.	*D. polypodioides* Blume	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropalar; polar ouline (excluding perine) transversely elliptical, sides convex; equatorial longitudinal view plano-convex to concave-convex; equatorial tranverse view, proximal view planar to concave, distal view convex; perinate. Size: E: 34.36(39.63)43.17± 2.33; P: 16.31(22.92)27.43±2.97. Laesura: concealed by wing-like perine. Perine: costate-alate, loosely reticulate; irregular envelope separated from exine surround the spore with costae forming loose irregular reticulation or wing-like muri, reticulation often incomplete, lacunae large irregular polygons, 3–9 µm across; wing-like muri project 1–7µm, terminating margins entire; surface or perine smooth under SEM. Specimens examined. Java: East Java, Mt.Wilis, T.Ng. Praptosuwiryo 1582, 1604, 1612, 1651.
13.	*D. porphyrorachis* (Baker) Diels	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical, sides convex; equatorial longitudinal view (excluding perine) plano-convex; equatorial tranverse view, proximal, proximal face planar, distal convex; perinate. E: 39.89(46.39)50.98±3.53; P: 21.74(27.57)30.49±2.87. Laesura: concealed by perine. Perine: alate to costate-alate, alate sometimes anastomosing forming loose reticulation; irregular envelope, separated from exine, surrounds the spore in loose anastomosing wing, forming a very loose reticulation, reticulation often incomplete; lacunae very irregular polygons, 10–16 µm across; muri thin, wing-like muri project 1.5–9.0 µm; terminating margin ciliate or papillate; surface of perine holed, holes irregular and formed by fallen papillae. Specimens examined. Borneo: East Kalimantan, Bukit Bangkirai, T.Ng. Praptosuwiryo 930; Kutai, Mt. Baratus, W. Meijer 872.
14	*D. prescottianum* (Wall.) Moore	Monolete, bilaterraly symmetrical (made asymmetric by perine), heteropolar, polar outline (excluding perine) transversely elliptical; equatorial longitudinal view plano-convex; equatorial transverse view, proximal face planar, distal face concave; perinate. E: 32.72(39.11)51.44±5.16; P: 0.23(24.79)33.56±3.63. Laesura: concealed by perine. Perine: alate, costate-alate, loosely reticulate; irregular envelope separated from exine surrounds the spore in continuous anastomosing wings, forming a loose reticulation; lacunae large, irregular polygons 5–8 µm; thin wing-like muri project 4–9 µm, terminating margins ciliate or echinate. Exine: visible through perine, smooth-granulate under SEM. Specimen examined. Sumatra: J.v. Borssum W. s.n. (17–6–1953).
15.	*D. procumbens* Holtt.	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical; equatorial longitudinal view (excluding perine) plano-convex; equatorial transverse view, proximal face convex, distal face hemispherical; perinate. Size: E: 43.03(53.78)63.20±4.72; P: 29.52(33.22)37.31±2.39. Laesura: concealed by perine. Perine: alate under LM, costate under SEM, irregular envelope separated from exine surrounds the spore in irregular, wing-like costae, often anastomosing to form a loose reticulation, lacunae shallow irregular polygons 8–22 µm, muri 1.6–6.6 µm, surface of muri and lacunae smooth. Specimens examined. Malay Peninsula: Pahang, R.E. Holttum 36503. Java: West Java, Mt. Salak, T.Ng. Praptosuwiryo 1455.

Table 1. Continued

No.	Species	Spores description & specimen examined
16.	*Diplazium* sp. 1	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline elliptical, sides convex; equatorial longitudinal view concave-convex to plano-convex; equatorial transverse view, proximal face planar to concave, distal face hemispherical; perinate. E: 36.44(44.06)63.70±8.92; P: 23.89(29.04)43.07±5.73. Laesura: concealed by perine ridge. Perine: micro costate, costae broken, reticulate irregularly, densely echinate under SEM, echinae single elements irregularly apart and projecting 1.1–2.1 µm. Exine: often visible through perine, smooth. Specimen examined. Java: West Java, Mt. Halimun, T.Ng. Praptosuwiryo 1798.
17.	*D. simplicivenium* Holtt.	Monolete, bilaterally symmetrical, heteropolar; polar outline elliptical, sides convex; equatorial longitudinal view plano-convex to concave-convex to plano-convex; equatorial transverse view, proximal face planar to concave, distal face hemispherical; perinate. E: 43.99(50.61)54.60±3.36; P: 23.31(29.42)33.78)±2.79. Laesura: concealed by perine ridge. Perine: alate to costae alate, irregular envelope separated from exine surrounds the spore in irregular and in continuous or discontinuous anastomosing wing, forming a loose reticulation; lacunae large irregular polygons, occasionally with granulate deposite within; thin wing-like muri projected 3–8µm, terminating margins are almost entire. Exine: smooth regulate under SEM. Specimen examined. Java: West Java, Mt. Pangrango, T.Ng. Praptosuwiryo 1250.
18.	*D. silvaticum* (Bory) Sw.	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar, polar outline (excluding perine) transversely elliptical; equatorial longitudinal view (excluding perine) concave-convex; equatorial transverse view, proximal face concave, distal face hemispherical; perinate. E: 31.59(41.31)49.30±3.82; P: 18.64(23.56)26.41±2.46. Laesura: concealed by perine. Perine: alate under LM, costate-alate under SEM, irregular envelope separated from exine; alae form loose reticulation; lacunae irregular polygons c. 6.6–20.0 µm across, interior of lacunae showing micro irregular reticulate/fenestreta under SEM; muri very thin, wing-like, terminating margin irregularly echinate. Exine: visible through perine, smooth-granulate under LM. Specimen examined. Java: West Java, Bogor Botanic Gardens, T.Ng. Praptosuwiryo 1300 (Wild Fern).
19.	*D. speciosum* Blume	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical, sides convex-straight; equatorial longitudinal view (excluding perine) concave-convex; equatorial tranverse view, proximal face convex, distal face biconvex; perinate. E: 29.98(39.29)47.25±5.53; P:17.37(21.69)25.83±2.56. Laesura: concealed by perine ridge. Perine: alate, costate-alate, irregular envelope separated from exine; alae occasionally form loose reticulation; lacunae irregular polygons c. 6.6–10.8 µm across, interior of lacunae smooth under SEM; terminating margins of wing-like muri entire. Exine: rugulate, rugulae large, shallow, irregular anastomosing under SEM after separating from perine. Specimens examined. Java: West Java, Mt. Gede-Pangrango, T.Ng. Praptosuwiryo 1242; Mt. Halimun, T.Ng. Praptosuwiryo 1805.
20.	*D. spiniferum* Alderw.	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical, side convex; equatorial longitudinal view concave-convex to plano-convex; equatorial transverse view, proximal face planar to convex, distal face hemispherical; perinate. E: 31.96(39.52)47.77±4.18; P: 14.87(23.92)29.21±4.14. Laesura: concealed by perine. Perine: micro-costate, densely echinate under SEM, costae broken, densely micro reticulate, coralline; irregular envelope, separated from exine, surrounds the spore in densely coralline with enchinae project 1.5–2.3 µm; lacunae are very small, irregular polygons less than 0.5–1.5 µm across; heavily fenestrate throughout under SEM, giving coralline appearance. Exine: visible through perine. Specimen examined. Borneo: East Kalimantan, Pa Urar, Krayan, M.Kato, M. Okamoto & E.B. Walujo B10800.

Table 1. Continued

No.	Species	Spores description & specimen examined
21.	*D. sorzogonense* Presl	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical, sides convex; equatorial longitudinal view (excluding perine) plano-convex; equatorial transverse view, proximal face planar to convex; distal face hemispherical; perinate. E: 27.80(33.03)37.90±3.91; P: 13.95(20.92)26.39±3.82. Laesura: concealetd by perine. Perine: alate, costate-alate, irregular envelope separated from exine; lacunae irregular polygons c. 8.3–16.6 µm across; terminating margins of costae or wing-like muri entire. Exine: smooth under SEM. Specimen examined. Java, West Java, Mt. Halimun, T.Ng. Praptosuwiryo 1713.
22.	*D. subpolypodioides* Blume	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar; polar outline (excluding perine) transversely elliptical, sides convex-straight; equatorial longitudinal view (excluding perine) concave-convex; equatorial tranverse view, proximal face convex, distal face biconvex; perinate. E: 32.49(40.29)47.17±4.08; P: 17.15(21.54)28.28±2.90. Laesura: concealed by perine ridge. Perine: alate, costate-alate, irregular envelope, separated from exine; costae or alae sometimes form loose reticulation; lacunae irregular polygons c. 3.3–12.5 µm, interior of lacunae smooth under SEM; muri, wing-like, smooth, terminal margin entire. Exine: smooth under SEM. Specimens examined. Java: West Java, Mt. Gede, T.Ng. Praptosuwiryo 1335; East Java, Mt. Wilis, T.Ng. Praptosuwiryo 1605.
23.	*D. subserratum* (Blume) Moore	Monolete, bilaterally symmetrical, heteropolar; polar outline elliptical, sides convex; equatorial longitudinal view concave-convex to plano-convex; equatorial transverse view, proximal face planar to concave, distal face hemispherical; perinate. Size: E: 27.88(41.19)45.49±5.03; P: 16.54 (27.13)20±5.03. Laesura: concealed by perine ridge. Perine: costate-alate, irregular envelope, separated from exine; costae form irregular loose reticulation; lacunae irregular polygons c.to 23 µm across; interior of lacunae and costae smooth. Exine: visible throught perine, smoothly granulate under LM. Specimens examined. Java: West Java, Mt. Halimun, T.Ng. Praptosuwiryo 1704, 1705.
24.	*Diplazium* sp.2	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropalar; polar outline (excluding perine) transversely elliptical, sides convex; equatorial longitudinal view (excluding perine) plano convex, equatorial transverse view, proximal face planar, distal view convex; perinate. Laesura: concealed by wing-like alae spinous coralline perine. Perine: alate to costate alate, alae or costae forming irregular reticulation, reticulation often incomplete; lacunae with echinae and coralline within, wing-like muri project 3–11 µm, terminating margins pappilate or echinate; pappilae project c.0.7–1.5 µm; surface of perine echinae-coralline with densely irregular hole, windows-like. Exine: not visible through perine under SEM. Specimen examined. Java: West Java, Mt. Gede, T. Ng. Praptosuwiryo 1178.
25.	*D. tomentosum* Blume	Monolete, bilaterally symmetrical, heteropolar; polar outline elliptical, sides convex, equatorial longitudinal view plano-concex-concave convex, equatorial transverse view, proximal face planar to concave, distal face convex; perinate. E: 34.64(41.06)47.19±3.88; P: 23.14(26.77)28.67±2.13. Laesura: concealed by perine. Perine: alate to costate-alate, loosely reticulate; irregular envelope separated from exine surrounds the spore in continuous anastomosing wings, forming a loose reticulation, occasionally wing-like alae only form 3 reticulation surraound the spore; lacunae large irregular polygons, papillae within; thin wing-like muri projected 3–12 µm, terminating margin are often ciliate, ciliae projected to ca. 0.5 µm. Exine: visible through perine, granulate under LM. Specimens examined. Borneo: East Kalimantan, Bukit Bangkirai, T.Ng. Praptosuwiryo 827, 829, 911.
26.	*D. vestitum* Presl	Monolete, bilaterally symmetrical (made asymmetric by perine), heteropolar, polar outline (excluding perine) transversely elliptical, sides vonvex; equatorial longitudinal view (excluding perine) concave-convex; equatorial tranverse view, proximal face convex, distal face convec to hemispherical; perinate. E: 28.21(32.58)35.33±2.26; P: 16.55(19.94)22.73±1.64. Laesura: concelated by perine. Perine: costate-alate, almost no reticulation irregular envelope, separated from exine, surrounds the spore with alae-costae ridge (occasionally) projected 2–5 µm; surface of perine smooth. Exine: visible through perine, smooth under SEM. Specimen examined. Borneo: East Kalimantan, West Kutai, Tabang, M. Kato & H. Wiriadinata B 6097.

and *D. malaccense*. The variation of wing-like muri ornamentation, such as its reticulation, lacunae formed, projection, and terminating margins, differentiate among species. Spores of *D. tomentosum* and *D. malaccense* are similar. The two species would differ in the projection of wing-like muri. The first species is with wing-like muri projecion up to 14 µm, and the second with projection of wing-like muri up to 8 µm. *D. cordifolium* differs from other species of this group in its incomplete reticulation and large lacunae (up to 16 µm or more across). Spores of *D. simplicivenium* differs from those of *D. megasegmentum* in the existence of granulate deposit in lacunae. The first has granulate deposit within lacunae, while the second has no granulate deposit in the lacunae.

(7). Group VII. Spores that are decorated in echinate perine and non-fenestrate are found in *Diplazium* sp.1. Echinae is only a single element and its projection size is diversed. Density and size projection of echinae would be very useful for differentiating species in this group. Echinate spores under LM were also showed in *D. subsinuatum* and *D. virescens* of Thailand (Tseng-Chieng 1981). The projection of echinae was also useful to differentiate the two species, echinae of *D. subsinuatum* spores were longer than those of *D. virescens*.

(8). Group VIII. *Diplazium spiniferum*, *D. lomariaceum*, *D. porphyrorhachys* have spores with coralline, echinate and fenestrate sculptures. Three species can be differentiated by its more detail perine sculptures. *D. spiniferum* shows densely corraline with echinae project 1.5–2.3 µm with very small lacunae (0.5–1.5 µm). *D. lomariceum* has large lacunae (13–21 µm) with wing-like muri projection 3–10 µm. The projections of wing-like muri of *D. porphyrorachis* (1.5–9.0 µm) and its across size of irregular lacunae polygons (10–16 µm) are overlapping with those of *D. lomariceum*. Morphologically, the two last species are very similar.

(9). Group IX. Spores that have form alate-echinate and fenestrate are found in *Diplazium* sp.2. In a glance, this spore type is similar to those of *Diplazium* sp.1. However *Diplazium* sp.2. spores would be differentiated from *Diplazium* sp.1. spores with its micro lacunae sculpture. The lacunae decorated with coralline and densely irregular windows.

Diplazium spores of Thailand observed by Tseng-Chieng (1981) using LM revealed alate to costate-alate sculpture in eight species, viz. *D. dilatatum*, *D. squamigerum*, *D. wichurae*, *D. megaphyllum*, *D. mettenianum*, *D. petri*, *D. phaeolepis* and *D. doederleinii*. More detail of the perine surface can not be seen, so that the comparison between these costate-alate species and costate-alate species of Malesia could no be done. This is because Tseng-Chieng (1981) did not provide the spores figures from SEM.

This study indicates that perine characteristics are very important in supporting delimitation of species *Diplazium* observed. So that a tentative key for species of *Diplazium* observed is given.

Phylogenetic Analysis

The evolutionary trends presented by Van Konijnenburg-van Cittert (1999) can not be applied in plylogenetic relationship in *Diplazium* because they appear suitable to be implicated to genus level and above. All derived spore characters states summed up by Konijnenburg-van Cittert (1999) are exist in *Diplazium* (see Table 1.) All species of *Diplazium* usually reveal 32 and 64 spores per sporangium for apogamous and sexual, respectively. Therefore the direction of the characters state transformation was determined by method of outgroup comparison. Table 2 shows the characters and characters states utilized in phylogenetic analysis of *Diplazium*. The rooting of the inferred phylogenetic relationship revealed in Figure 1 was conducted by selecting spore description of *Athyrium filix-femina* cited from Large & Braggins (1991).

The 17 spore morphological characters (Table 2 and 3) were analysed to determine relationship among the 26 species of West Malesian *Diplazium*. The 100 equally most parsimonious trees of 131 steps produce a consistency index (CI) = 0.53 and retention index (RI) = 0.50.

Figure 5 showed tree number 1 of 100 the most parsimonius trees.However the strict consensus of 100 the most parsimonious trees resulted a polytomy tree with very low supported Boostrap value (Fig. 6). Separation between the ingroup and outgroup was also without a high supported Bootsrap value.

The results showed that there is an incongruence between the grouping generated from gross morphological data and those generated from spore morphology. As showed in Figure 6, the concensus tree of 100 most parsimonius tree, relationship among the species of *Diplazium* based on spore morphology are polytomy and elusive. Although topological of tree number 1 of 100 most parsimonious trees showed differentiation among 27 species analyzed (Fig. 5), however, it did not give

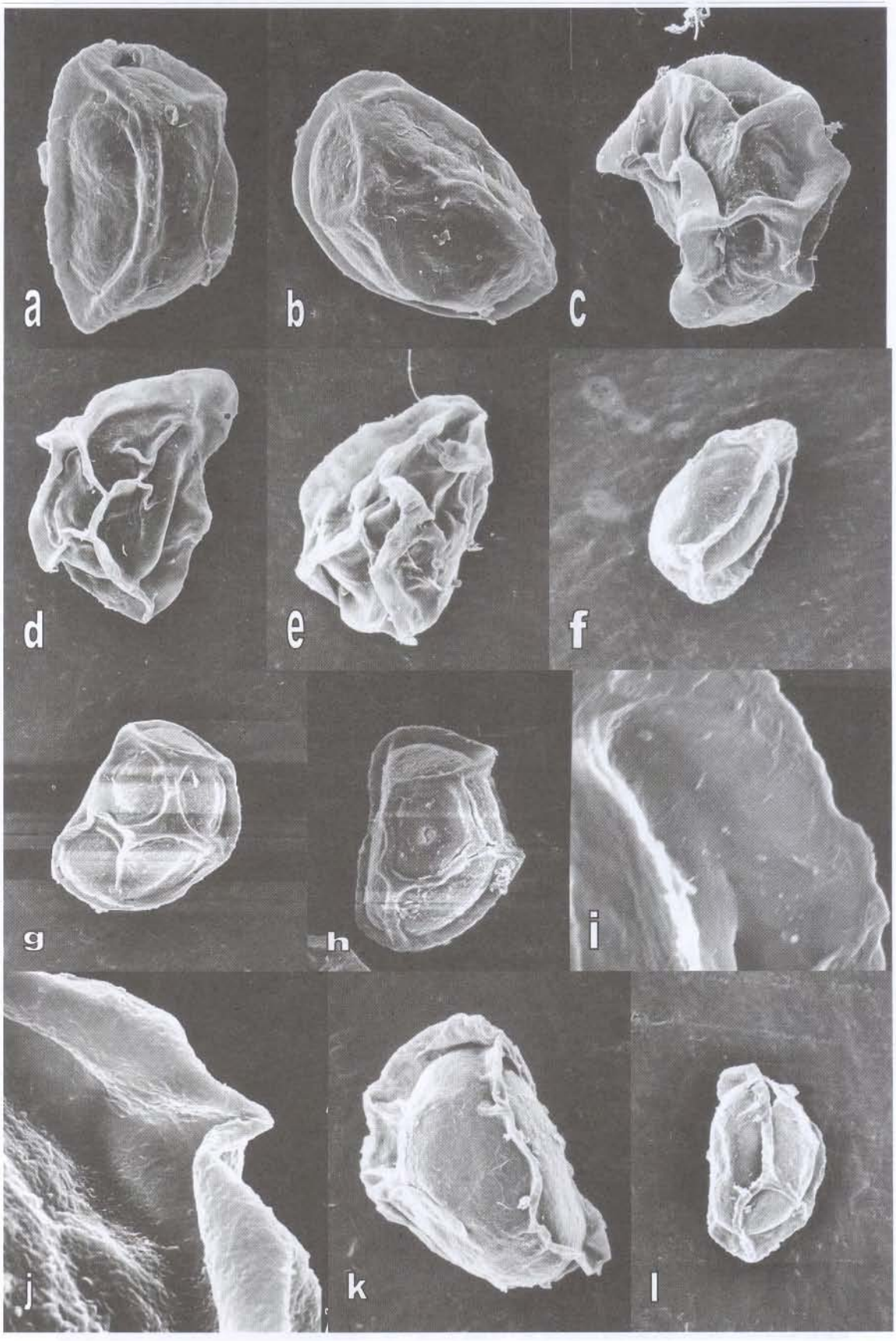

Fig. 1. Group I. a and b. *D. accedens*; c and d. *D. bantamense*; e. *D. lobbianum*; f and g. *D. pallidum*; h-j. *D. procumbens*; k and l. *D. sorzogonense*. Bar = 15 µm for a, b, f; Bar = 30 µm for c, d, e, g, h, and k. Bar = 3 µm for i and j.

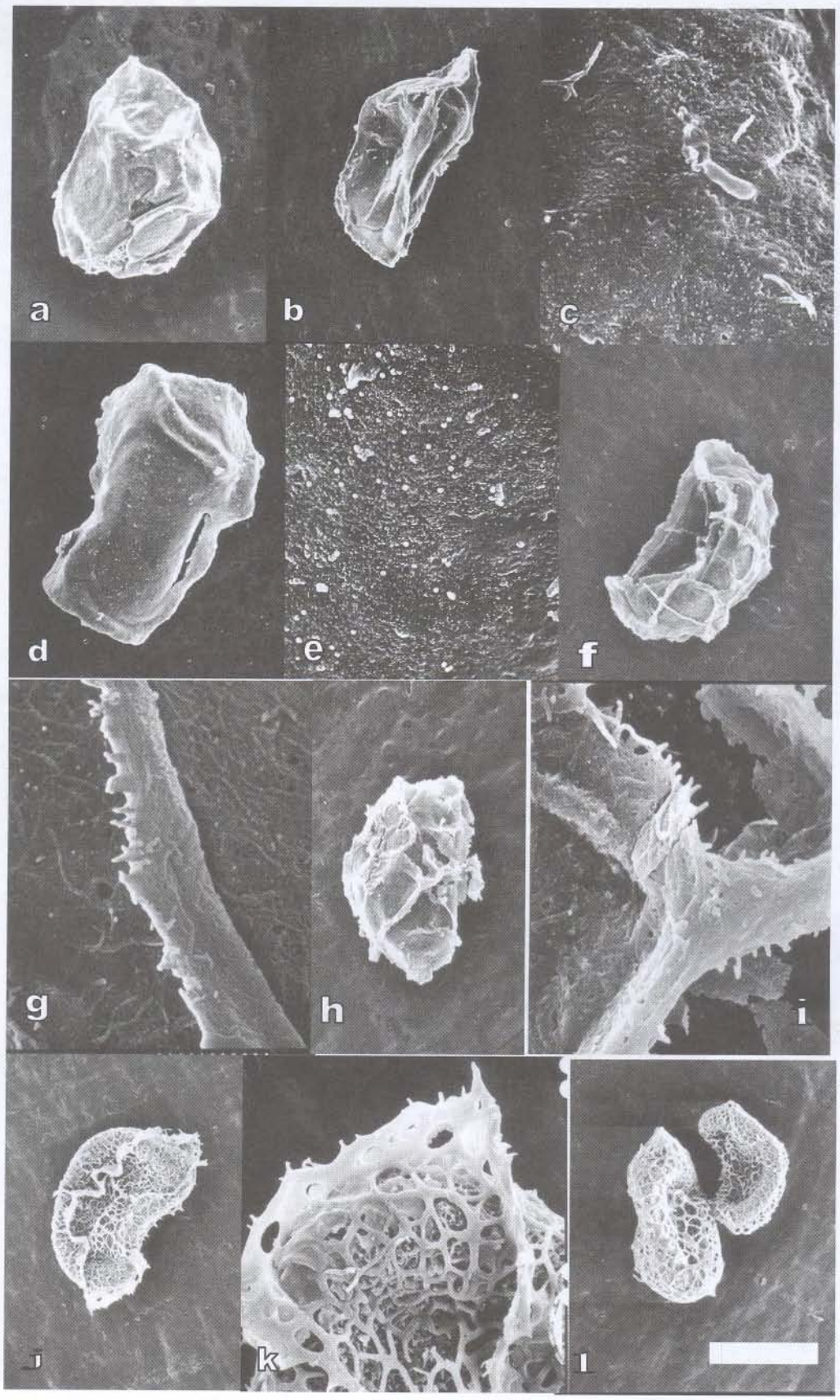

Figure 2. Group II. a. *D. subserratum*; b and c. *D. vestitum*; d and e. *D. vestitum* var. *borneense*; Group III. f and g. *D. crenatoserratum*; h and i. *D. prescottianum;* Group IV. j – l. *D. silvaticum*. Bar = 30 µm for a, b, d, f, h, j and l. Bar = 3 µm for c, e, g, i, and k.

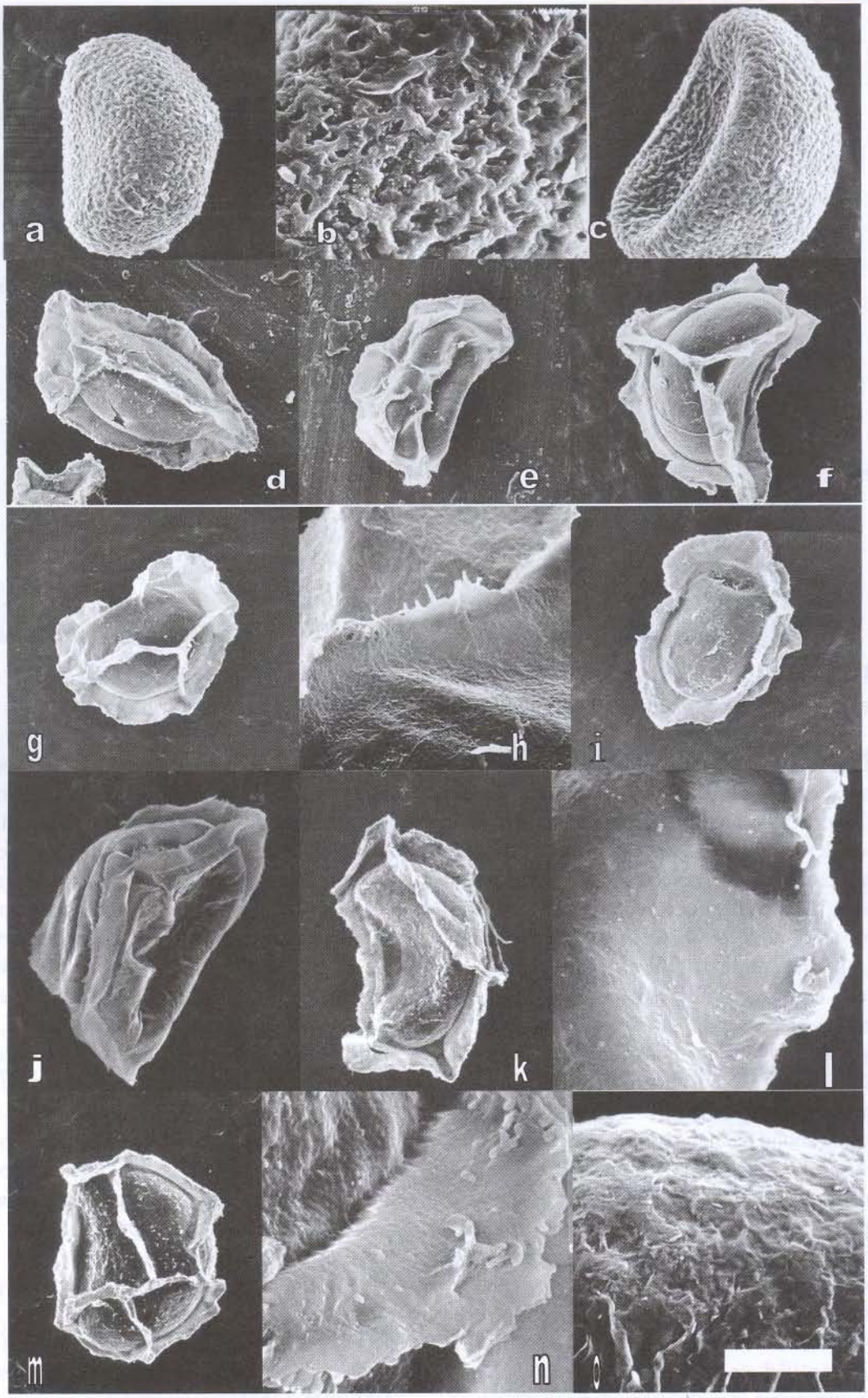

Figure 3. Group V. a–c *D. pallidum;* Group VI. d and e. *D. cordifolium*; g–i. *D. tomentosum*; j. *D. malaccense*; k and l. *Diplazium* sp.3; m–o. *D. simplicivenium*. Bar = 30 µm for a, c, d, e, f, g, i, j, k and m. Bar = 3 µm for b, h, l, n, and o.

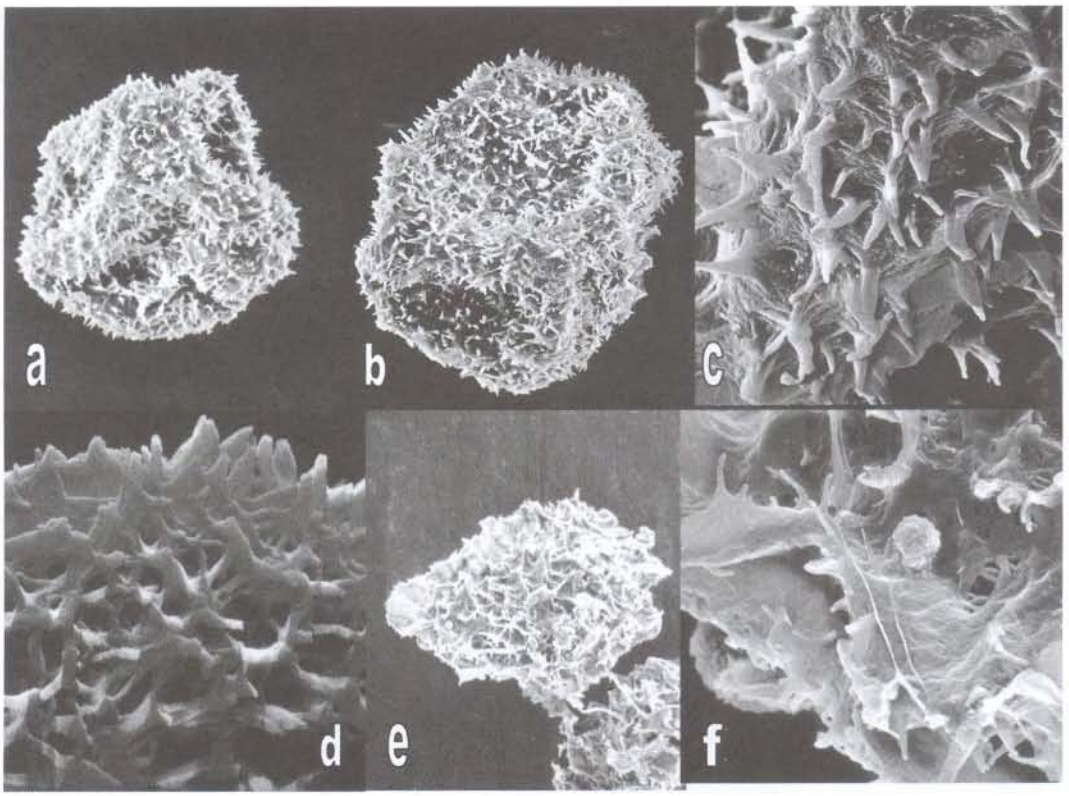

Figure 4. Group VII. a-c. *Diplazium* sp.1; Group VIII. d. *D. spiniferum*; Group IX. e and f. *Diplazium* sp. 2. Bar = 30 μm for a, b, and e. Bar = 3 μm for c, d, and f.

any logical relationships between the two closely related species.

Figure 5 showed that both the main clades and sub-clades are formed by the grouping of species that are not closely related species based on the gross morphological evidences. For example, *D. porphyrorachis* and *D. lomariaceum* are closely related. In the clade II, the two species were separated, the first species was in the same dichotomous branch with *D. cordifolium* and the second species was forming dichotomous branch with *D. prescottianum*. Based on gross morphological characters, *D. porphyrorachis* and *D. lomariaceum* shoud be in the same clade, meanwhile *D. prescottianum* should be forming a dichotomous branch with *D. pallidum*. The affinities of the two pairs species based on gross morphological characters are more reasonal. Morphologically, *D. porphyrorachis* and *D. lomariceum* share characters combination as follow: rhizome short-erect, wiry black roots; fronds narrowly elliptic, deeply pinnatifid or pinnate, apex coadunate, segments numerous, usually very dark green when living; scales abundant on stipe and rachis, narrow, usually dark and shiny; veins free. *D. cordifolium* has characters as follow: lamina simple and imparipinnate; simple ones oblong subdeltoid, 23–30 cm long, 8–13 cm wide above base, widest 1/3 from base, base cordate, apex acuminate, margin entire; imparipinnate ones nearly oblong deltoid in outline, 28–45 cm long, 24 cm wide; lateral pinnae 1–6 pairs, upper smaller, usually terminal ones largest, oblong subdeltoid, to 13–18 by 4–8 cm; upper pinnae sessile, broadly cuneate at base, lower pinnae shortly stalked, the largest 16 by 5.5 cm, cordate at base, apex acuminate, margin entire; texture coriaceous; veins at about 45–70°to costa on pinnate ones, 50–80° to midrib on simple ones, forked close to midrib, lower branch forked again 2–5 times, anastomousing irregularly about 1/3–1/2 from margin. *D. pallidum* and *D. prescottianum* share characters combination as follow: scales dark brown, lineary subtriangular, margin entire; lamina simply pinnate, terminal pinnae conform to lateral pinnae; pinnae linear; veins free, twice or thrice forked near costa.

Incongruence between two independent or more data in phylogenetic analysis of ferns occasionally occurs. Dubuisson et al. (1998) has evaluated the interaction between three independent data sets (anatomy/morphology, cytology and molecules) within the ferns genus *Trichomanes* (*Hymenophyllaceae*). It was revealed that the data sets showed high and significant level on incongruence.

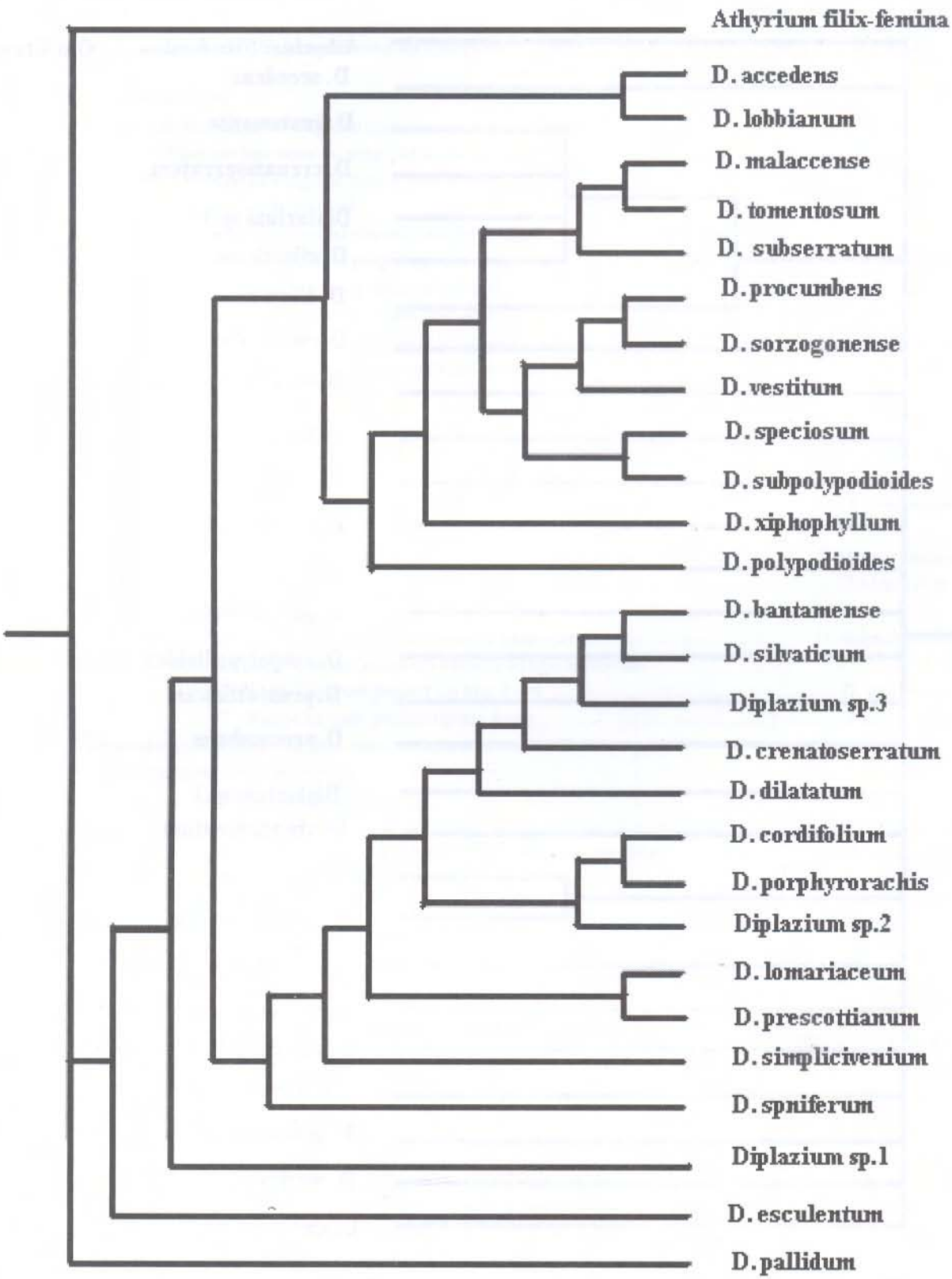

Figure 5. Tree number 1 of 100 the most parsimonius trees

The illogical relationship of the spore topological tree result in this study also indicates that superficial similarity of mature perispores may not be the best indicator of systematic relationship among species. This indication was also showed by Ranker (1989) in the study on spore morphology of new world *Hemionitis*, *Gymnopteris* and *Bommeria* (*Adiantaceae*). Ranker (1989) presumed that similar surface patterns of spore may be derived through different development pathways.

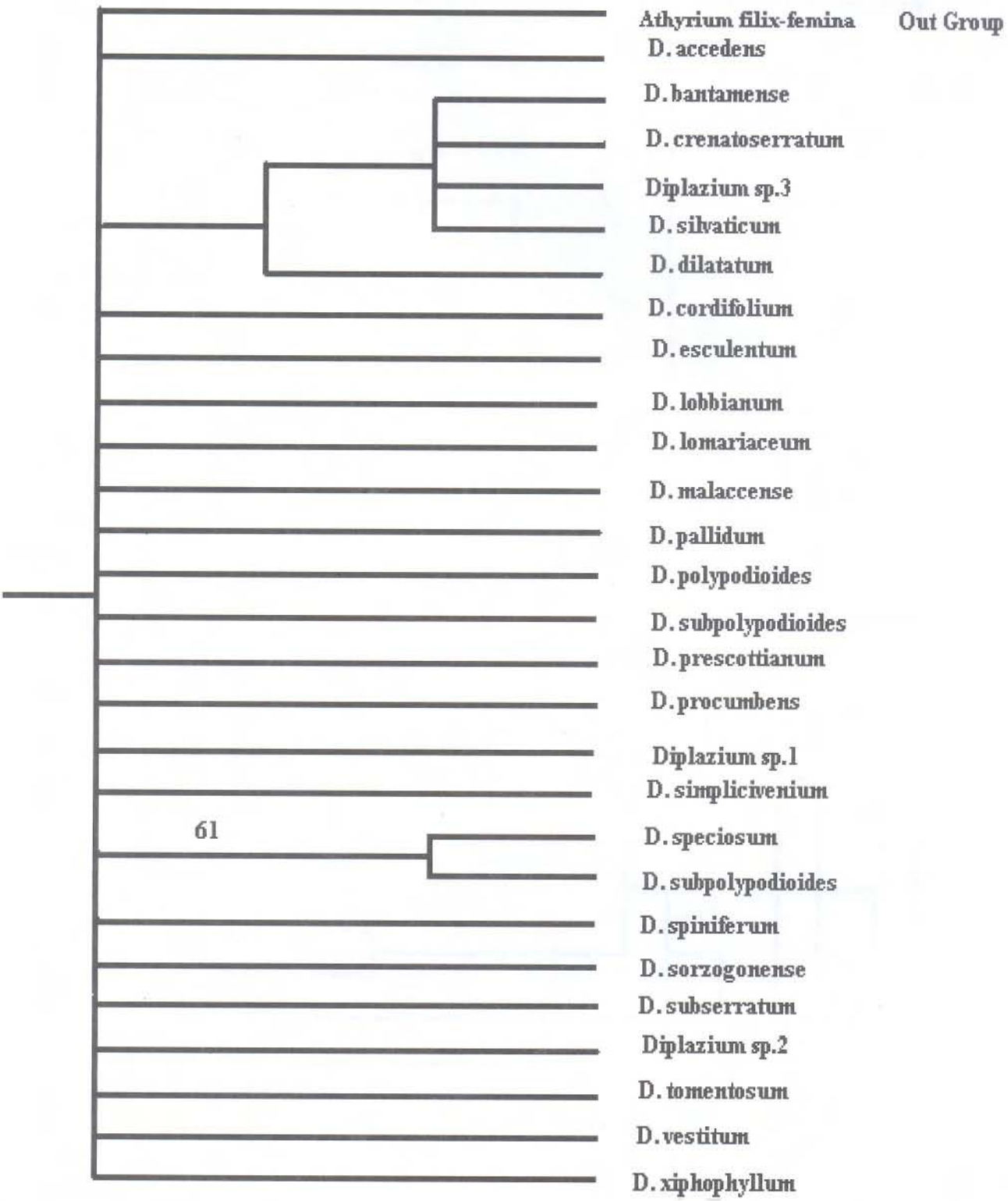

Figure 6. The strict consensus of 100 the most parsimonious trees. The number above line showed supported the Boostrap value.

ACKNOWLEDGEMENTS

The first author greatly appreciates to Prof. Dr. Mien A. Rifai for his advice, guidance and encouragements throughout her Ph.D course at Institute Pertanian Bogor (IPB). She also expresses her gratitude to Prof. Dr. Ryoko Imaichi, who offered the chance and funding to carry out spore morphology observation by using LM and SEM at the Chemical and Biological Department, Japan Women's

Key to the species of *Diplazium* based on spores morphological characters observed by using SEM

1. Perine alate to costate-alate
   2. Perine coralline, fenestrate on wing-like muri ..... *D. silvaticum*
   2. Perine not coralline, not fenestrate on wing-like muri
      3. Terminating margins of wing-like muri or costae almost entire
         4. Reticulation often incomplete
            5. The mean of equatorial size over 40 µm or more
               6. Wing-like costae project up to 13 µm; exine smooth under LM ..... *D. bantamense*
               6. Wing-like costae project up to 5 µm; exine granulate under LM ..... *D. pallidum*
            5. The mean of equatorial size less than 40 µm
               6. Lacunae (irregular polygons) 10 µm or more across ..... *D. accedens*
               6. Lacunae (irregular polygons) 9 µm or less across ..... *D. polypodioides*
         4. Reticulation complete
            5. Perine alate
               6. Lacunae with granulate deposit within ..... *D. simplicivenium*
               6. Lacunae without granulate deposit within
                  7. Exine regulate ..... *D. lomariceum*
                  7. Exine smooth
                     8. Wing-like muri project up to c. 7 µm ..... *D. procumbens*
                     8. Wing-like muri project up to c. 13 µm ..... *Diplazium* sp. 3.
            5. Perine costate-alate
               6. Surface of perine (lacunae) smooth granulate ..... *D. subpolypodioides*
               6. Surface of perine (lacunae) smooth irregular fibrous
                  7. Wing-like muri project up to c. 4 µm ..... *D. speciosum*
                  7. Wing-like muri project up to c.6 µm ..... *D. sorzogonense*
      3. Terminating margins of wing-like muri or costae echinate or ciliate
         4. Reticulation often incomplete
            5. Lacunae up to 12 µm across
               6. Surface of perine smooth or scarcely micro papillate; not coralline ..... *D. xiphophyllum*
               6. Surface of perine densely sharp echinae or spine; coralline ..... *Diplazium* sp.2
            5. Lacunae up to 16 µm or more across
               6. Perine not forming holes from fallen ciliae ..... *D. cordifolium*
               6. Perine forming holes from fallen ciliae
                  8. Like-fiber materials on muri not forming continuous lines ..... *D. porphyrorachis*
                  8. Like-fiber materials on muri forming continuous lines ..... *D. crenatoserratum*
         4. Reticulation complete
            5. Terminating margins of wing-like muri almost entire or smooth ..... *D. subserratum*
            5. Terminating margins of wing-like muri ciliate
               6. Costae or alae usually not forming reticulations ..... *D. vestitum*
               6. Costae or alae forming many reticulations
                  7. Perine alate
                     8. Wing-like muri project up to 9 µm
                        9. Wing-like muri with irregular holes ..... *D. prescotianum*
                        9. Wing-like muri without irregular holes
                           10. Wing-like muri project up to 8 µm ..... *D. malaccense*
                           10. Wing-like muri project 8 < x < to 15 µm ..... *D. lobbianum*
                     8. Wing-like muri project 9 < x < 14 µm ..... *D. tomentosum*
                  7. Perine costate-alate
                     8. Lacunae without irregular holes within ..... *D. dilatatum*
1. Perine rugulate or ciliate to coralline-echinate
   2. Perine rugulate ..... *D. esculentum*
   2. Perine ciliate to coralline-echinate
      3. Not fibrous-coralline, not fenestrate ..... *Diplazium* sp.1.
      3. Thick fibrous-coralline, fenestrate ..... *D. spiniferum*

Table 2. Characters, character states, and coding for 17 characters utilized in construction of spore morphology dataset of *Diplazium*.

	(0)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1. Equatorial longitudinal view	concave-convex	plano-convex	biconvex	{01}						
2. Proximal face	concave	planar	convex	{01}	{12}					
3. Distal face	hemispherical	convex	concave	biconvex						
4. The Equatorial size mean	31.47–32.37	32.58–34.24	34.25–38.18	38.19–39.63	40.29–42.15	2. 44.06	45.69–47.39	47.96	50.61–51.42	53.78–53.88
5. The Polar size mean (μm)	19.51–19.94	20.80–2154	21.69–22.92	23.48–24.57	24.88–26.28	26.37–27.57	28.55–29.04	29.42	31.48–33.22	
6. The existence of perine fold	no fold	with fold								
7. Perine fold type	costate	costate-alate	alate	echinate	{12}					
8. End of ridge	rounded	slightly crested	{01}							
9. The existence of coralline	not exist	exist								
10. Type of perine fold reticulation	incomplete reticulate	complete reticulate	{01}							
11. The widest lacunae	1.50–2.50	2.60–4.60	4.70–6.70	6.80–10.7	10.8–12.5	10.90–18.5	18.6–20.3	20.4–23.7	23.8–26.1	
12. Fold of Crest Margine	Smooth/entire	Waved	Papillate	Ciliate	Echinate	{01}	{23}	{24}	{34}	
13. The longest projection of wing like muri or costae	1–2.1	2.9–4	5	6–7	8	9	10–11	12	13	15
14. Perine surface	Smooth	Fibrous-like	Ciliate/papilate	Holed	Echinate	Small granulate	{05}			
15. Existence of microscopic holes on perine	Absent	Present								
16. Exine appearance under SEM	Smooth	Granulate	Rugulate	{01}						
17. Exine appearance under LM	Smooth	Granulate	Rugulate	{01}						

Table 3. Coding for 17 characters utilized in construction of spore morphology dataset of *Diplazium*.

Species	Characters and Characters States																
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Athyrium filix-femina	3	3	0	2	4	1	0	0	0	0	0	0	0	5	0	2	0
D. accedens	3	3	0	0	0	1	1	0	0	0	0	0	3	0	0	0	0
D. bantamense	0	0	0	9	8	1	1	1	0	0	9	0	8	0	0	0	0
D. cordifolium	1	1	1	4	5	1	3	2	0	1	7	4	4	0	0	0	0
D. crenatoserratum	0	0	1	3	3	1	2	1	0	0	6	2	4	1	1	0	0
D. dilatatum	0	0	1	6	6	1	3	1	0	0	0	3	9	0	0	0	0
D. esculentum	3	3	0	3	5	0	-	0	-	-	0	-	0	0	0	1	2
D. lobbianum	3	3	0	6	4	1	1	1	0	0	0	6	-	2	0	0	0
D. lomariaceum	2	1	0	3	3	1	3	1	0	-	0	5	6	0	0	2	2
D. malaccense	3	0	1	1	1	1	3	2	0	1	0	3	4	0	0	0	1
Diplazium sp. 3.	0	2	1	8	8	1	2	1	0	0	0	0	8	0	0	-	-
D. pallidum	1	2	0	7	8	1	0	0	0	0	6	0	2	0	0	1	0
D. polypodioides	3	3	1	3	2	1	1	2	0	0	3	0	3	0	0	1	0
D. porphyrorachis	1	1	1	6	5	1	3	1	0	0	7	0	5	0	0	0	0
D. precottianum	1	1	2	3	3	1	3	1	0	1	3	8	4	0	0	0	3
D. procumbens	1	2	0	9	9	1	2	2	1	0	8	5	3	0	0	0	0
Diplazium sp.1	3	3	0	5	6	1	0	1	0	0	0	4	0	4	0	0	0
D. simplicivenium	3	3	0	8	7	1	3	1	0	2	0	0	4	6	0	3	0
D. silvaticum	0	0	0	4	3	1	2	1	0	0	7	4	-	0	0	3	0
D. speciosum	0	2	3	3	1	1	3	1	0	0	4	0	-	0	0	2	0
D. spiniferum	3	4	0	3	3	0	1	1	0	0	0	-	0	0	0	0	0
D. sorzogonense	1	4	0	1	1	1	3	2	0	0	6	0	-	0	0	0	0
D. subpolypodioides	0	2	3	4	1	1	3	2	0	0	4	0	-	0	0	0	0
D. subserratum	3	3	0	4	1	1	1	2	0	1	8	0	-	0	0	-	0
Diplazium sp.2.	1	1	1	-	-	1	3	1	1	0	0	7	-	4	1	-	-
D. tomentosum	3	3	1	4	5	1	3	2	0	1	0	3	7	0	0	1	0
D. vestitum	0	2	3	1	0	1	1	0	0	0	0	0	2	0	0	0	0
D. xiphophyllum	3	4	1	4	4	1	3	2	0	0	3	2	6	0	0	0	0

University, Japan. She thanks to Dr. Irawati and Dr. Eko Baroto Walujo for their advices and technical support. She indebted to Dr. Mami Konomi, Dr. Mamiko Sato, Dr. Chie Tsutsumi, Mie Hashino and Rieko Fujinani for their useful helps and technical supports for this research in Japan.

REFERENCES

Blacmore S. 2000. *The palynological compass: the constribution of palynology to systematics*. In: Nordenstam B, El-Ghozaly, Kassas M. (Eds). Plant systematics for the 21st century. Pp 161–177. London: Portland Press.

Copeland EB. 1947. *Genera Filicum*. MASS, USA: Waltham.

Dubuisson J-Y, Hebant-Mauri R & Galtier J. 1998. Molecules and morphology: Conflicts and congruence within the fern genus *Trichomanes* (*Hymenophyllaceae*). *Mol. Phylog. & Evol.* 9 (3): 390–397.

Ferrarini E, Ciampolini F, Pichi Sermolli REG & Marchetti D. 1986. Iconographia palynologica Pteridophytorum Italiae. *Webbia* 40(1): 1–202.

Harris WF. 1955. *A manual of the spores of New Zealand Pteridophyta*. Department of Scientific and Industrial Research Bulletin 116. Government Printer, Wellington.

Kato M. 1977. Classification of *Athyrium* and allied genera of Japan. *Botanical Magazine* (Tokyo) 90: 23–40.

Konijnenburg-van Cittert van JHA. 1999. On the evolution of fern spore anchitecture. In: Kurmann MH & Hemsley AR (Eds). *The Evolution of Plant Architecture*. Pp 179–287. Kew: Royal Botanic Garden.

Kramer KU, Holttum RE, Moran RC & Smith AR. 1990. *Dryopteridaceae*. In: Kramer KU and Green PS (Eds.). *Pteridophytes and Gymnosperms*. Springer-Verlag, Berlin. pp 101–144.

Large MF & Braggins JE. 1991. *Spore atlas of New Zealand Ferns and Ferns allies*. SIR Publishing, Wellington.

Ohta N & Takamiya M. 1999. Taxonomix studies of the *Diplazium mettenianum* complex (*Woodsiaceae*; *Pteridophyta*) in Japan: Morphology, cytology and taxonomy of plants with normal-shaped spores. *J. Plant. Res.* 122: 67–86.

Pettit JM. 1966. Exine stucture in some fossil and recent spores and pollen as revealed by light and electron microscopy. *Bull. Brit. Mus. (Nat. Hist.) Geol.* 13: 221–257.

Ranker TA. 1989. Spore morphology and generic delimitation of new world *Hemionitis*, *Gymnopteris*, and *Bommeria* (*Adiantaceae*). *Amer. J. Bot.* 76(2): 297–306.

Roos M. 1995. Flora Malesiana: progress, needs and prospects. In: J. Dransfield et. al.(Eds.) *Plant Diversity in Malesia III*. Proceedings of the Third International Flora Malesiana Symposium.Pp. 231–246.

Roubik DW. 2003. Pollen and spores of Barro Colorado Islands. 2003. http://striweb.si.edu/roubik/ [24 Feb. 2007].

Swofford DL. 1998. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sinauer Associates, Sunderland, Massachusetts.

Takamiya M, Takaoka C & Ohta N. 1999. Cytological and reproductive studies on Japanese *Diplazium* (Woodsiaceae; Pteridophyta): Apomictic reproduction in *Diplazium* with evergreen bi- to tripinnate leaves. *J. Plant. Res.* 112: 419–436.

Tagawa M. 1972. Ferns of Borneo collected by M. Hirano and M. Hotta 6. *Acta Phytotax Geobot.* 25 (2–3): 61–68.

Tseng-Chieng H. 1981. *Spore flora of Taiwan*. National Taiwan University, Taiwan.

Tryon AF & Lugardon B. 1991. *Spore of the Ptridophyta*. New York: Springer Verlag.