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Systematic Study on Davalliaceae in Peninsular Malaysia

Haja Maideen Kader Maideen

Doctor of Philosophy
The University of Edinburgh
Royal Botanic Garden Edinburgh
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Abstract

Davalliaceae is a fern family established by A. B. Frank in 1877, based on the genus *Davallia*. It contains about 150 species in 8-12 genera and is restricted to the Old World tropics and subtropics. They are mostly epiphytes with long creeping fleshy rhizomes covered with peltate scales. In Peninsular Malaysia, the Davallioid ferns belong to *Davallia* Sm., *Humata* Cav., *Leucostegia* C. Presl and *Araiostegia* Copel. (Parris & Latiff, 1997). This study used morphological, cytological and molecular (three chloroplast regions) data in an attempt to classify Davalliaceae, especially in Peninsular Malaysia. The results presented in this thesis showed moderate to strong support for the paraphyly of genera in Davalliaceae, especially in Peninsular Malaysia. The results were incongruent with the latest classification based on morphology (Nooteboom, 1998) but congruent with a global study based on molecular data. The phylogeny showed that *Leucostegia* doest not belong to Davalliaceae. Four major clades were recognised in Davalliaceae, namely the *Araiostegia* Clade (AC); *Davallia* with two clades: *Davallia* Clade I (*denticulata* clade and *dimorpha-divaricata* clade), *Davallia* Clade II (*scyphularia-solida* clade and *trichomanoides* clade) and the *Humata* clade (HC). Maximum parsimony and Bayesian analyses of *rps4* + *rps4-trnS* IGS and combined three regions produced congruent topologies, but the topologies of *rbcL* and *trnL-F* region produced only slight differences. The expanded *rbcL* data also showed that all species were fully resolved without having a separated/regional clade. In general the molecular phylogenetic reconstruction of Davalliaceae based on *trnL-F* and *rps4-trnS* regions showed additional support that none of the genera in this family is monophyletic. However, the results produced could have been interpreted with more confidence if species from *Davallodes* (not reported in Peninsular Malaysia) and species from other parts of the world were included in the study. Most genera of Davalliaceae in the study were characterized as having a single base chromosome number, x = 40. All plants studied were diploids except for *Davallia vestita* which was polyploid (hexaploid). Spores of Davallioid ferns are monolete (ellipsoid) and have verruculate ornamentation. Phylogenetic reconstruction based on morphology was inconclusive and character evolution in the genus and related genera was characterised by homoplasy. However, the topology of the tree based on morphological characters showed *Davallia* spp. and *Humata* spp. in different lineages. The morphological characters which support separation of *Humata* and *Davallia* were inconclusive and were limited to the presence of a white waxy rhizome and the shape of the indusium (shell shape). *Araiostegia* should remain as a separate genus as, based on molecular data, it forms a clade sister to *Davallia*. *Leucostegia* should be excluded from Davalliaceae as the molecular data showed the genus more closely linked to the outgroup. Consequently Davalliaceae in Peninsular Malaysia comprises fifteen species in two genera, *Araiostegia* (1 species) and *Davallia* with 14 species in three sections.

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Chapter One

Introduction

1.1 General

Pteridophytes, or ferns and fern allies, are seed free vascular plants distributed by spores. They mostly prefer warm and moist environments. Over the last century, this group has been classified in many ways. In the 18th century, all the classifications were based on annulus, sporangium and structure of indusium. In 19th century, the classification based on morphology continued with further addition of characters such as position and shape of sori, sporangium and indusium, vegetative characters, anatomy and venation. The rapid development in scientific research in the 20th century marked the turning point in the field of pteridology as new classifications were based on methods other than anatomy and morphology. These include knowledge of palynology, cytology, phytochemistry and, more recently molecular phylogeny. Recently, the application and the introduction of molecular sequence data has enhanced our potential to interpret pteridophyte evolution and phylogenetic relationships within groups.

The improvement of pteridological knowledge especially in genomic studies has contributed to a decrease in the number of taxa. Pichi-Sermoli (1977) classified ferns into 64 families and 443 genera. In 1992 Brummitt classified this group into 41 families and 328 genera and the latest classification by Smith et al. (2006), who classified all the extant ferns, recognised 37 families. The recent studies (e.g.

Pryer et al., 2001) have confirmed that Pteridophytes are not a monophyletic group; some of the so-called 'fern allies', e.g. *Psilotum* and *Equisetum* nest within the fern clade, whereas the Lycophytes: *Isoetes*, *Selaginella* and *Huperzia* form a separate monophyletic group, the Lycophytina. Ferns and seed plants form another monophyletic group, the Euphyllophytina.

1.2 Davalliaceae A. B. Frank

The family Davalliaceae or davallioid ferns is one of 37 families of ferns (Smith et al., 2006). They are commonly known as Hare's foot ferns, reflecting the characteristic rhizomes. They are mostly epiphytes with long creeping fleshy rhizomes covered with peltate scales. The frond is simple to pinnatifid, firmly herbaceous to coriaceous, veins free, usually forked, false veins sometimes recurrent from the sinuses. The fertile fronds are often more contracted than the sterile fronds, sometimes more dissected. Sporangia are borne in small discrete sori terminal on the veins, on very short side veins or dorsal on the veins, submarginal or sometimes medial, indusiate with the indusium cup-shaped and opening towards the margin, attached at the base and sometimes at the sides. Davalliaceae in Palaeotropical region is represented by 8-12 genera (Brummitt, 1992). However in Malaysia up to six genera (*Araiostegia, Davallia, Davallodes, Humata, Leucostegia* and *Scyphularia*) are represented by 24 species. Out of these only 17 species in five genera (*Araiostegia, Davallia, Humata, Leucostegia* and *Scyphularia*) have been reported in Peninsular Malaysia (Parris and Latiff, 1997).

The classification of this family has attracted the attention of several taxonomists since the 20th century as several fundamental problems relating to its taxonomy, especially generic delimitation, still remain inconsistent. The latest classification of pteridophytes in the Malesian region by Nooteboom (1998) lumped all *Araiostegia, Humata and Scyphularia* species within *Davallia*. However a global molecular study by Tsutsumi and Kato (2005) based on the chloroplast *rbcL* region placed species of *Araiostegia, Humata* and *Scyphularia* in a different clade from the *Davallia*.

1.3 Study area

Malaysia is situated in Southeast Asia and consists of two areas: Peninsular Malaysia, on the Asia mainland and the states of Sabah and Sarawak, on the island of Borneo. It has an area of 329,750 km^2. Peninsular Malaysia is the extension of continental Asia southward from Indochina which divides the Andaman Sea and the Straits of Malacca from the South China Sea. It comprises an area of 131,587 km^2. It is bordered by Thailand in the north and Singapore in the south (Figure 1). The area is an important phytogeographic area defining the floristic region known as western Malesia (John, 1995). All of the offshore islands that are part of the territory of the eleven states that make up Peninsular Malaysia are included. The climate of Peninsular Malaysia is equatorial, hence it is hot and humid throughout the year. Average daily temperatures vary from about 21° to 32° C and rainfall averaging 250 cm annually. The climate is dominated by two monsoon wind systems, with the Northeast Monsoon blowing between

November and March and the Southeast Monsoon blowing from June to September.

1.4 Objective of study

The objectives are as follows:

- To make a taxonomic revision of the family Davalliaceae in the study area.
- To produce detailed descriptions of davallioid ferns, based on morphological and molecular data and, where possible, field study.
- To infer the phylogeny of the davallioid ferns in Peninsular Malaysia
- To explore speciation and evolution in epiphytic ferns

This study is designed to understand and clarify the following:

- The diversity of Davalliaceae of Peninsular Malaysia, producing a comprehensive and accurate list of species of this family within this area.
- Phylogeny of Davalliaceae

This study is expected to complement molecular data on the family from other parts of the world, and will clarify the relationships between the species and genera of this family.

1.5 Thesis structure

In order to facilitate the understanding and objectives outlined above, this thesis is divided into seven chapters. The first chapter provides a general introduction to ferns, Davalliaceae and information of the study area. The second

chapter is a bibliographic review of Davalliaceae with particular emphasis on its classifications from the 19th century to the present time. A morphological study which examines the characters used in the classification and the use of morphological characters in phylogenetic study is discussed in chapter three. The fourth chapter examines chromosome counts of specimens from the study area. Chapter five is a molecular systematic study of Davalliaceae based on data from *rbcL*, *trnL-F* and *rps4-trnS* chloroplast DNA regions to construct phylogenetic trees. Morphological character mapping and evolution of epiphytism will be discussed in chapter six. Chapter seven is a general discussion and conclusion.

Figure 1: Map of Peninsular Malaysia. The red dots show the locality of samples collected for molecular and cytological studies. See appendix II for details of species location.

Chapter Two

Bibliographic review of Davalliaceae

2.1. History of family Davalliaceae

The family Davalliaceae was established by A. B. Frank in 1877 based on the genus *Davallia*, named after Edmund Davall, a Swiss botanist. The taxonomic status of the davallioid ferns at the species, genus and family levels is confusing. The origin and evolutionary lineages are not well understood and this circumstance clouds the systematic position of the group. In the 19th century, botanists lumped the davallioid ferns together with many unrelated genera into the family Polypodiaceae (e.g. Diels, 1902; Ching 1940). This unnatural family consist of about 7,000 species grouped on the basis of a single morphological character, the vertical annulus (Dickason, 1946).

In 1902, Diels modified the traditional family Polypodiaceae based on soral and indusial characters. He divided the family into nine tribes of which six were subdivided into two subtribes, and one into four subtribes. One of the tribes (created by Diels) was the Davallieae with fifteen genera (*Arthropteris, Nephrolepis, Humata, Saccoloma, Diella, Leptolepia, Davallia, Microlepia, Odontosoria, Wibelia, Dennstaedtia, Monochosorum, Schizoloma, Dictyoxiphium* and *Lindsaya*).

Christensen (1938) placed the davallioid ferns in the Davallioideae of the family Polypodiaceae. He considered the subfamilies of his Polypodiaceae to be well characterized, and suggested that these subfamilies could be better considered as families. In 1940, Ching raised the rank of Christensen's subfamilies and some tribes to family level. Christensen's subfamily Davallioideae became the family Davalliaceae, divided into subfamilies Davallioideae and Nephrolepioideae. Ching grouped his families in a series of supposed evolutionary lines and placed his Davalliaceae in the Lindsayoid-Davallioid series.

In 1946, Dickason published his `Phylogenetic Study of the Ferns of Burma' in which he analyzed the phyletic patterns of variation among the ferns. The outcome of that study was a system of classification in which the families of ferns were grouped into five orders. The largest of the orders was the Filicales with 42 families arranged into 12 groups according to soral position. These families agree very closely with those of Ching. He placed Davalliaceae in group J (according to his scheme), characterized by sori each subtended on the basal side by a scale-like or halfcup-like indusium. However, this system of classification has been criticized by Pichi-Sermolli (1959) for being based on only one character (soral position).

In 1947, Holttum proposed a new system of classification of the polypodiaceous ferns. Later, Holttum (1949) published a more complete scheme that included all the leptosporangiate ferns. He recognized fourteen

families, and one of them, the Dennstaedtiaceae, was subdivided into eleven subfamilies, one of which is the Davallioideae (consisting of six genera: *Araiostegia, Leucostegia, Davallodes, Davallia* (including *Trogostolon, Scyphularia* and *Parasorus*), *Humata* and *Rumohra*. In 1947 Copeland published his classic *Genera Filicum* which has been for many years one of the best reference books in fern classification. He recognized three orders and 21 families, nineteen of which formed the order Filicales. The family Davalliaceae as presented by Copeland was a group of twelve genera (*Araiostegia, Leucostegia, Davallodes, Trogostolon, Davallia, Scyphularia, Humata, Parasorus, Oleandra, Nephrolepis, Arthropteris* and *Psammiosorus*). Alston (1956) proposed a new classification based on spore morphology, chromosome number, stipe anatomy and the presence of hairs and scales on the rhizome. The Davalliaceae of Alston consist of four genera viz. *Nephrolepis, Arthropteris, Oleandra* and *Davallia.*

In 1975, Crabbe & Jermy proposed a new generic sequence of pteridophytes for use in herbaria in which the subfamilies Davallioideae and Olendroideae were included in Davalliaceae. They included eleven genera (*Humata, Trogostolon, Scyphularia, Parasorus, Davallia, Davallodes, Paradavallodes, Araiostegia, Leucostegia, Gymnogrammitis* and *Rumohra*) under Davallioideae.

Pichi-Sermolli (1977) revised his taxonomic scheme and provided an enumeration of all the genera of living pteridophytes. His family Davalliaceae

consist of ten genera which subdivided into three groups, two being monotypic (*Leucostegia* and *Gymnogrammitis*). The remaining group consist of eight genera (*Humata, Trogostolon, Scyphularia, Parasorus, Davallia, Davallodes, Paradavallodes* and *Araiostegia*).

Kato (1985) in his study of the genera of Davalliaceae divided the family based on anatomy and morphology of rhizome, dermal appendages, leaf architecture, venation and indusia into nine genera (*Araiostegia, Davallia, Davallodes, Gymnogrammitis, Leucostegia, Pachypleuria, Parasorus, Scyphularia* and *Trogostolon*). In 1990, Kramer classified Davalliaceae into five genera, namely *Araiostegia, Leucostegia, Davallodes* (including *Paradavallodes*), *Davallia* (including *Humata, Pachypleuria, Scyphularia, Parasorus* and *Trogostolon*) and *Gymnogrammitis*. A summary of the classification of Davalliaceae is given in Table 2.1.

2.2 Research on Davalliaceae

There have been several taxonomic studies of Davalliaceae in some geographic regions, e.g. Malesia (Nooteboom, 1992, 1994, 1998). A few systematic studies on genera of Davalliaceae have been done for example in *Davallodes* (Copeland, 1927; Holttum, 1972), *Araiostegia* and *Davallia* (Sen et al., 1972) and *Humata* (de Joncheere, 1977). Several systematic studies have focused on morphology and anatomy (Perez Arbeláez, 1928; Sen et al., 1972; Phillips and White, 1967; Kato, 1975; Nayar and Bajpai, 1977; Kato and Mitsuta, 1980); sorus and spore ornamentation (Kato, 1975; 1985; and

Rodl-Linder and Nooteboom, 1997); gametophyte (Sen et al., 1972; Atkinson, 1973; Nayar and Bajpai, 1977); cytology (Manton, 1968; Lovis, 1977); phytochemistry (Richardson, 1983) and molecular systematics (Schneider et al., 2002; Tsutsumi and Kato, 2005).

2.3 Distribution

The distribution of this family with ca 150 species is restricted to the Old World tropics or subtropics. They occur in the extreme SW of Europe, in Macaronesia, tropical and South Africa and its islands, throughout South and East Asia north to Korea and Hokkaido, east to Tahiti and south to SE Australia and the extreme north of New Zealand, they are absent from Hawaii.

Table 2.1: Classification of Davallioid ferns

Diels (1902)	**Christensen (1938)**	**Ching (1940)**	**Holttum (1949)**
Family Polypodiaceae	Family Polypodiaceae	Family Davalliaceae	Family Dennstaedtiaceae
Tribe Davallieae	Subfamily Davallioideae	Subfamily Davallioideae	Subfamily Davallioideae
Genera	Genera	Genera	Genera
Arthropteris *Davallia* *Dictyoxiphium* *Diella* *Dennstaedtia* *Humata* *Leptolepia* *Lindsaya* *Microlepia* *Monochosorum* *Nephrolepis* *Odontosoria* *Saccoloma* *Schizoloma* *Wibelia*	*Arthropteris* *Davallia* *Davallodes* *Humata* *Leucostegia* *Nephrolepis* *Psammiosorus*	*Acrosphorus* *Davallia* *Davallodes* *Gymnogrammitis* *Humata* *Leucostegia* *Paradavallodes* *Parasorus* *Scyphularia* *Trogostolon*	*Araiostegia* *Davallia* *Davallodes* *Humata* *Leucostegia* *Rumohra*

Table 2.1: continued

Copeland (1947)	**Alston (1956)**	**Crabbe and Jermy (1975)**	**Pichi-Sermolli (1977)**
Family Davalliaceae	Family Davalliaceae	Family Davalliaceae Subfamily Davallioideae	Family Davalliaceae
Genera	Genera	Genera	Genera
Araiostegia *Arthropteris* *Davallia* *Davallodes* *Humata* *Leucostegia* *Nephrolepis* *Oleandra* *Parasorus* *Psammiosorus* *Scyphularia* *Trogostolon*	*Arthropteris* *Davallia* *Nephrolepis* *Oleandra*	*Araiostegia* *Davallia* *Davallodes* *Gymnogrammitis* *Humata* *Leucostegia* *Paradavallodes* *Parasorus* *Rumohra* *Scyphularia* *Trogostolon*	*Araiostegia* *Davallia* *Davallodes* *Gymnogrammitis* *Humata* *Leucostegia* *Paradavallodes* *Parasorus* *Scyphularia* *Trogostolon*

Table 2.1: cont.

Kato (1985)	**Kramer (1990)**	**Nooteboom (1998)**
Family Davalliaceae Genera *Araiostegia* *Davallia* *Davallodes* *Gymnogrammitis* *Leucostegia* *Pachypleuria* *Parasorus* *Scyphularia* *Trogostolon*	Family Davalliaceae Genera *Araiostegia* *Davallia* *Davallodes* *Gymnogrammitis* *Leucostegia*	Family Davalliaceae Genera *Davallia* *Davallodes* *Leucostegia*

Chapter Three

Morphology

3.1 Introduction

Morphology forms the basis of taxonomic descriptions and generally constitutes the most important data in delimiting and circumscribing taxa. All classical classifications were based on morphological characters. Morphological characters are features of external form which have been used long before anatomical evidence. They have been a primary source of taxonomic evidence since the beginning of plant descriptions as they are easily observed and can be used in keys and descriptions. Although morphological characters are widely used in fern classifications, only a few cladistic studies have been made using these characters (Hill and Camus, 1986). This probably reflects the lack of morphological characters in ferns and the widespread phenomena of polyploidy and hybridization which are common mechanisms of speciation in ferns. These have led many pteridologist to conclude that phylogeny reconstruction for ferns is an impossible task (Kramer and Tryon, 1990). However following an increasing number of molecular systematic studies in seed plants, pteridologists are now using molecular data for the construction of phylogenetic trees and using morphological characters to increase the number of characters used in study and as a tool for comparison (e.g. Pryer et al., 1995 and Dubuisson et al., 1998).

Copeland (1908, 1927) published the first system of classification of Davalliaceae based on morphology. He examined leaf form and cutting, leaf texture, scales, pubescence and form and size of sori to distinguish taxa in the family. Several minor modifications have been made by various authors since Copeland (Nayar and Bajpai, 1976; Sen and Sen, 1970; Nooteboom, 1992, 1994, 1998; Sen, 1992; de Joncheere, 1977; Kato, 1985; Sen, Sen and Holttum, 1971). The characters used in the infrageneric classification were rhizome structure (including roots and waxy layer around the rhizome), rhizome scales (attachment, shape, colour and indumenta), leave (arrangement, texture, and vein), location of the sori and attachment and shape of indusia.

Although the family has been revised by Nooteboom (1998), the taxonomy remains controversial with two distinct problems: reduction in number of genera and no of species as a large number of species has been lumped together in one genus. The present study is based on species of Davalliaceae which have been reported recently for Peninsular Malaysia by Parris and Latiff (1997).

3.2. **Materials and methods**

3.2.1 **Materials**

This study is based on the fresh specimens collected in the study area in June 2005 and the herbarium specimens from Universiti Kebangsaan Malaysia, Bangi (UKMB), Forest Research Institute of Malaysia (KEP), Singapore Botanical Garden (SING), Royal Botanic Gardens Kew (K), Natural History

Museum, London (BM), Royal Botanic Garden Edinburgh (E) and National Herbarium Leiden (L). See appendix II for list of specimens in this study.

3.2.2 **Methods**

3.2.2.1 **Describing morphology characters**

All significant morphological characters for example, rhizome (structure/size/surface), scales (attachments/shape/margin), fronds (type/texture/branching), system of vein (true or false vein/length), indusium (attachments/shape) and spores (shape and ornamentation) were examined by careful observation of all specimens (herbarium specimens and collected during field work) with the help of hand-lens, stereo microscope and scanning electron microscopy. Photographs were also taken to code these characters. Classification of the names and confirmation of name in herbarium specimens were made using published floristic accounts.

a. **Scanning Electron Microscopy (SEM)**

This method included three stages: mounting, coating and scanning the specimens.

Mounting specimen:

With stereo microscopy, the specimens (fronds, scales and spores) from species were selected and transferred by fine forceps or brush to 12 mm carbon discs mounted on 12.5 mm aluminium stubs.

Specimen coating:

The mounted specimens were transferred into the sputter coater chamber (Emitech) and were vacuumed. Argon gas was delivered for 30 seconds when the high vacuum status has been reached. The specimens were coated with gold palladium at a preset deposition rate of 25 mA for 3 minutes. The coating generates a conductive surface which prevents the build up of negative charge that would interfere with the images. The specimens were then ready to be scanned.

Specimen scanning:

The system was ventilated with nitrogen and then evacuated for several times before it was ready to use. Once ready the coated stubs were placed in the SEM chamber (LEO supra 55VP). Stubs were first scanned at low magnification to select a suitable frame and view. The working distance was between 8 and 11 mm and the scanning voltage (EHT) at 5 kV was set to the optimum resolution of the image. The magnification, focus and contrast/brightness were adjusted in scanning mode. The images were saved and the output was printed.

3.2.2.2 **Phylogenetic analysis**

a. **Coding morphological character**

The morphological matrix was compiled using the same 36 taxa as in the molecular study. A total of seventeen morphological characters from sporophytes were coded for seventeen species reported in study area. The coding for outgroup taxa was obtained from literature (Holttum, 1968). The

spore characters were based on personal observations from the SEM study and literature (e.g. Rödl-Linder and Nooteboom, 1997). However spore characters was omitted from the analysis because no variability was observed. All morphological characters used in the analysis, their states and the complete character matrix are given in Table 3.1 and Table 3.2.

b. **Analysis**

The analysis was performed with PAUP 4b.10 (Swofford, 2002). The phylogeny was reconstructed using maximum parsimony, heuristic search with 10,000 random addition replicates and TBR branch swapping. All characters were equally weighted and unordered. Equally most parsimonious tree from the analysis was summarized using strict consensus. Clades support was assessed by bootstrap analysis (Felsenstein, 1985).

3.3 **Results**

3.3.1 **Characters observation in specimens examined were**:

A. Rhizome (character 1 - 4)

The rhizome is always creeping and varies from short to long in length. All of the species studied are densely covered with scales, however *Leucostegia immersa* is covered in both scales and hairs. Hairs are not found in *L. pallida*. In the *Humata* group and *Davallia corniculata*, their rhizomes are also covered with a white waxy layer. Roots are found on the ventral side of lateral buds except *Leucostegia*, where roots are scattered on all sides of the rhizome (Figure 3.1).

B. Rhizome scales (character 5 - 8)

The scales which covered most of rhizome are variable in shape, colour and indumenta and could be used as a tool to differentiate between species. There are three types of scales attachment in Davalliaceae: basifixed scales, pseudopeltate and peltate scales. Basifixed scales with the broad base attached to rhizomes in *Leucostegia pallida* and often with hairs are found in *L. immersa.* In other genera the rhizome scales are either peltate or pseudopeltate (basally attached with a cordate overlapping base). Pseudopeltate scales are found in *Araiostegia hymenophylloides*, *D. divaricata* and *D. dimorpha*. The scales are acicular or nearly acicular, evenly narrowed towards the apex above the much broader base, or just evenly narrowed. In a number of species, apical and marginal scales have multicellular hairs (such as in *D. solida, Scyphularia triphylla*, and *Humata repens*), ciliate or toothed (for instance *D. denticulata, D. dimorpha* and *D. trichomanoides*) (Figure 3.2).

C. Stipe (character 9)

The stipe is articulated and a constant character in all species studied. All species have pale or dark brown colour, adaxially grooved, glabrous or a few scales especially on basal part and its length vary from 1mm (*Humata parvula*) to 60 cm long (*D. divaricata*) (Figure 3.3).

D. Frond (character 10 – 14)

The fronds of Davalliaceae are diverse from simple (*H. angustata* and *S. triphylla*) to pinnately compound with intermediate forms. Lamina are often

thin (*A. hymenophylloides,* and *Leucostegia*) and firm or coriaceous in texture (mostly in *Humata* spp), usually triangular and narrowed to the base. Veins are pinnately branched, free, ending behind the margin or reaching it. "False veins" are also present between the true veins in a few species (*D. corniculata, D. denticulata* and *D. trichomanoides*) (Figure 3.4)

E. Sori (character 15)

Sori are located terminally or at the end of a vein in *Leucostegia immersa, L. pallida, Humata parvula* and *Humata angustata*) while in other species the sori are located at the forking point of veins except in *A. hymenophylloides, H. pectinata* and *H. parvula* where the sori are found at the bending point of a vein (Figure 3.5).

F. Indusium (character 16 -17)

The indusium is a protective covering of the sporangia formed by an outgrowth from the frond. It is an important character which assists to distinguish the genera in Davalliaceae. The attachment of the indusium can be basal only (*A. hymenophylloides, H. angustata, H. pectina* and *H. repens*), basal and partially attached at the side (*H. heterophylla, H. parvula, H. vestita* and *Leucostegia pallida*) or at basal and side (*D. corniculata, D. denticulata, D. dimorpha, D. divaricata, D. solida. D. trichomanoides* and *S. triphylla*); the shape is reniform, shell shaped or pouch shaped (Figure 3.4 and 3.5).

Figure 3.1. Rhizome: a. hairy and scales; b. with scales only; c. white waxy rhizome; d. rhizome without white waxy layer; e. round shape; f. flattened shape.

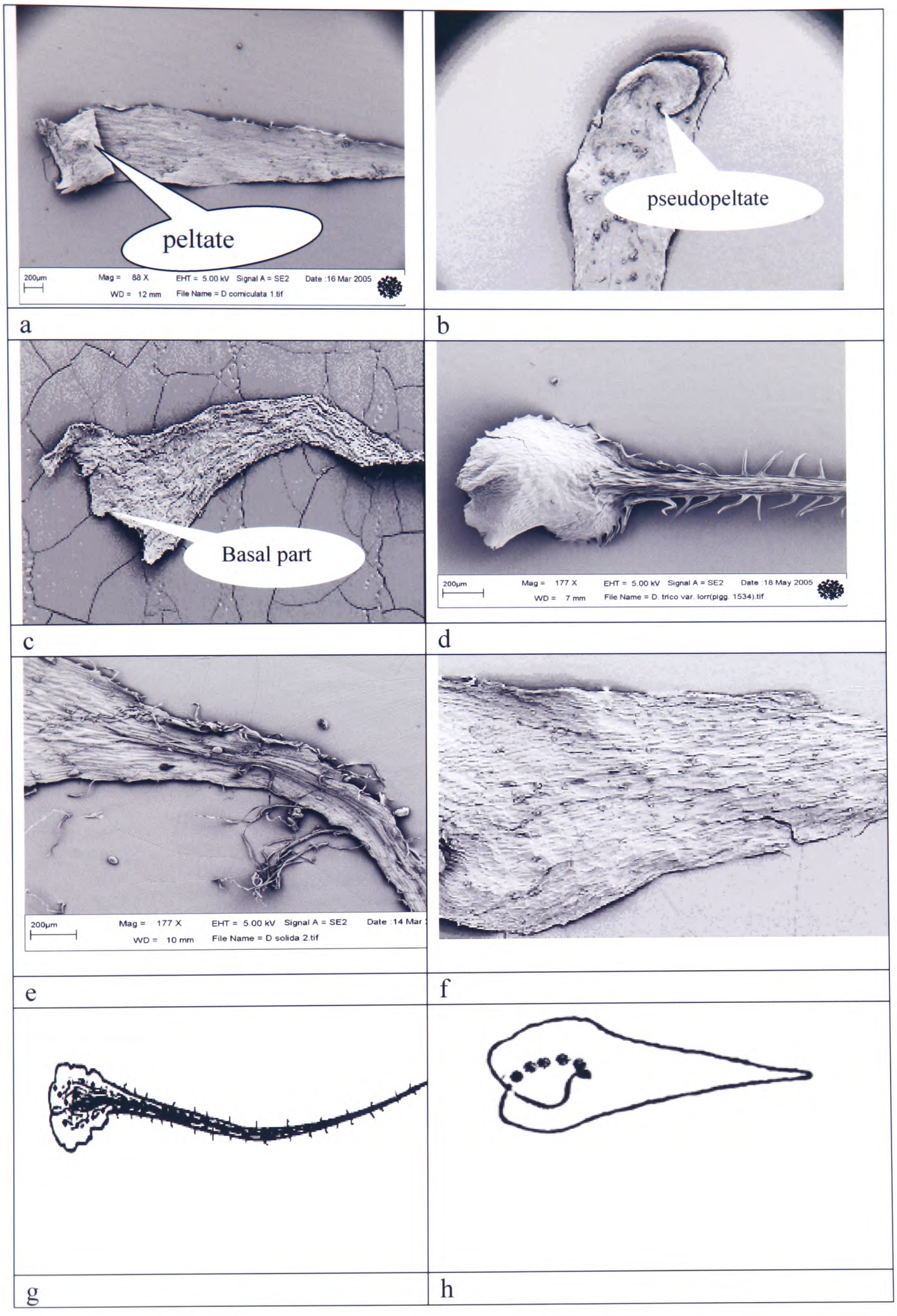
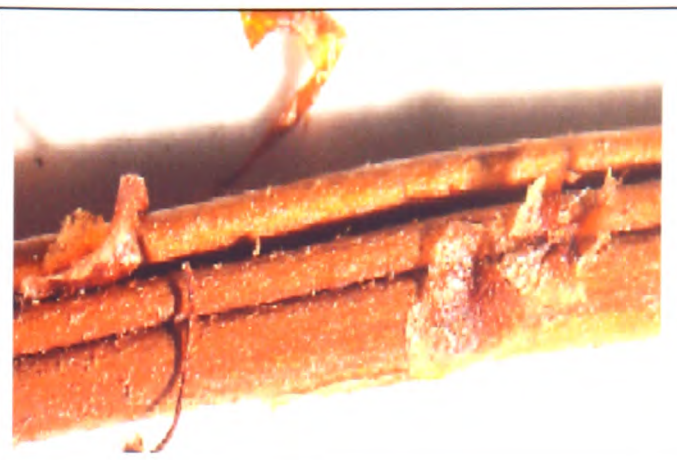


Figure 3.2. Scale characters: a. Peltate; b. pseudopeltate; c. basifixed; d. margin with teeth; e. margin with setae or multisetae; f. entire margin; g. acicular shape; h. non acicular shape

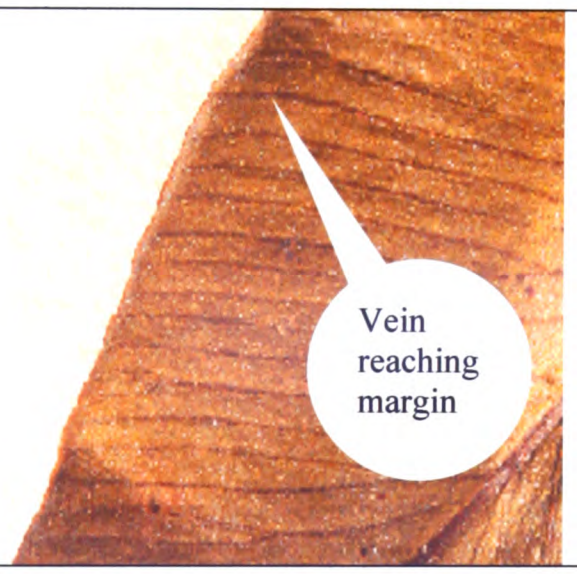
a



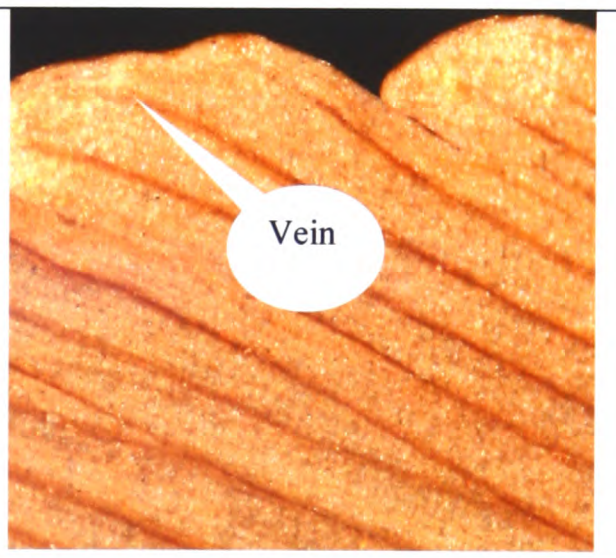
b

c

d



e.

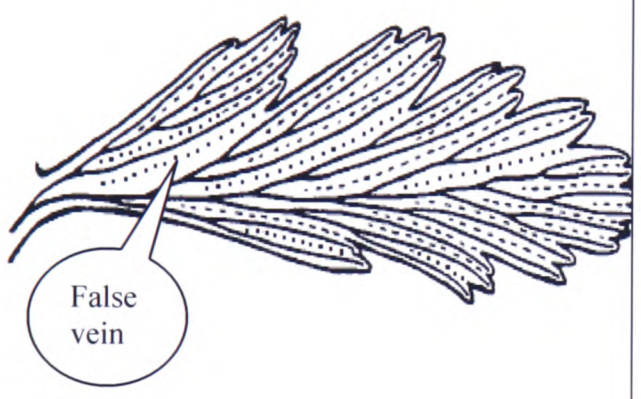


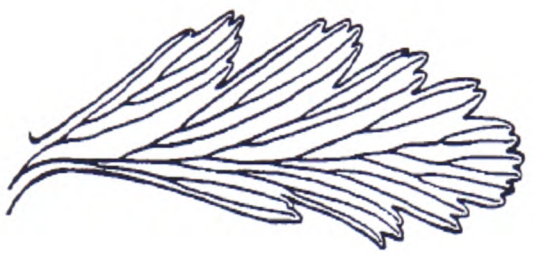
f

Figure 3.3. Frond and stipe: a. Glabrous stipe; b. stipe with scales; c. simple frond; d. bipinnate frond; e. vein reaching margin; f. vein not reaching margin

a

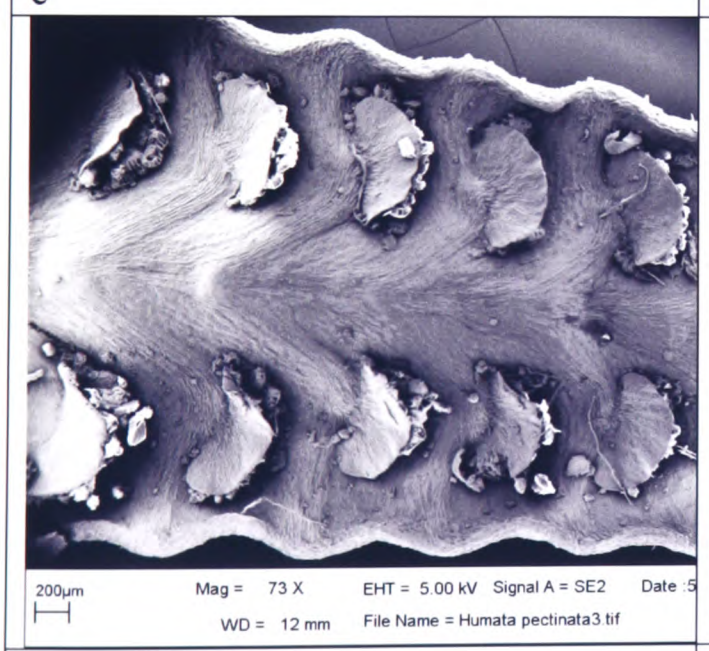
b

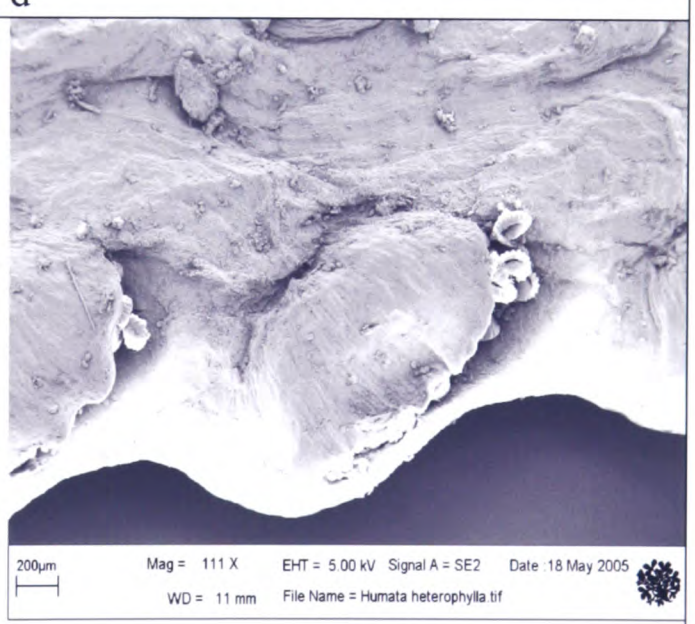




c

d

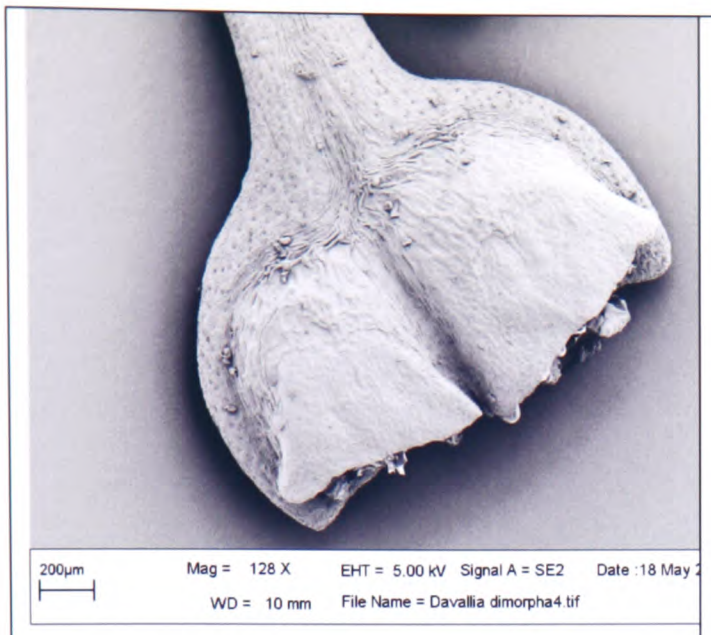




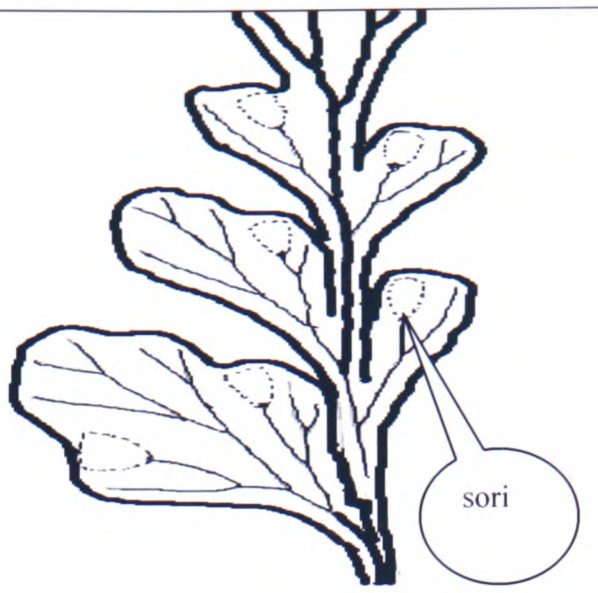
e

f

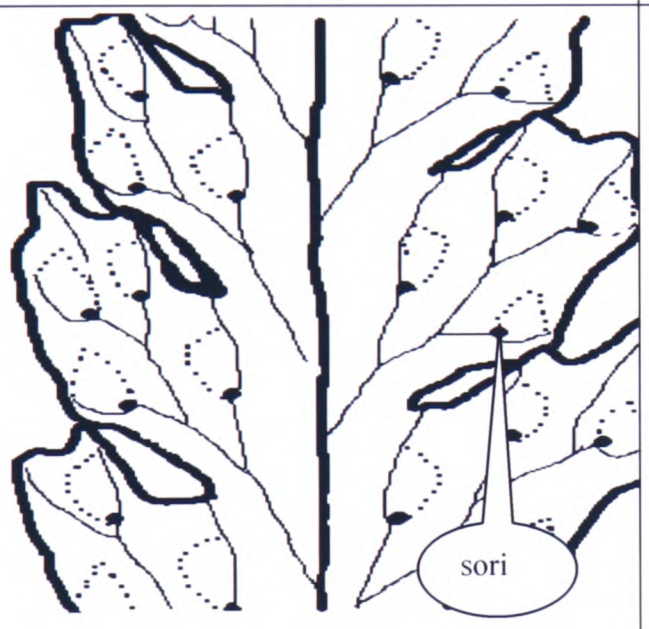
Figure 3.4. a. dimorphic fronds; b. frond not dimorphic; c. pinnae with false vein; d. pinnae without false vein; e. indusium with basal attachment; f. indusium with basal and half side attachment.



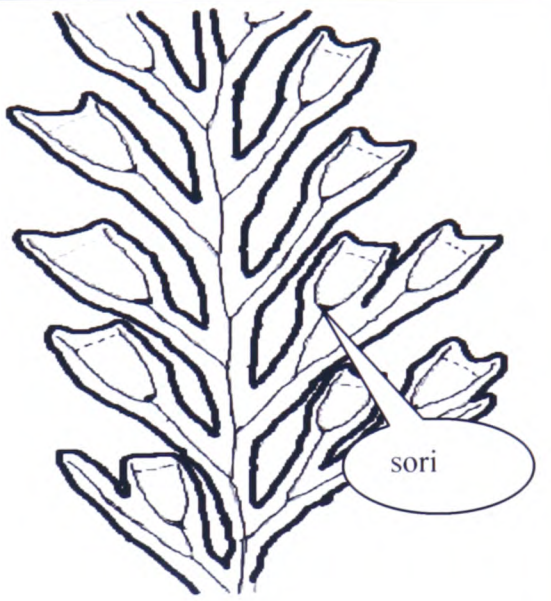
a



b



c



d

Figure 3.5. Indusium and location of sori: a. Indusium with basal and side attachment; b. sori at terminal position; c. sori at bending point; d. sori at forking point

Table 3.1: Morphological character and character state scored in present study.

Character	No. characters	Character state		
Rhizome				
	1. Outerlayer	0 = hairy & scale	1= scales only	
	2. Surface	0 = white waxy	1= not white waxy	
	3. Shape	0 = rounded;	1= flattened	
	4. Size	0 = diameter < 5 mm	1= > 5 mm	
Scales				
	5. Colour	0 = brown/red brown	1 = black	
	6. Attachment	0 = peltate	1= pseudopeltate	2= basifixed
	7. Margin	0 = have teeth	1= setae or multisetae	2 = non teeth/setae
	8. Shape	0 = acicular	1 = non acicular	
Stipe				
	9. Surface	0 = glabrous	1 = with a few scales	
Frond				
	10. Structure	0 = coriaceous	1 = herbaceous	
	11. Type	0 = simple/pinatifid	1 = bipinnate – quadripinnate	
	12. Venation	0 = reaching margin	1 = not reaching margin	
	13. Structure	0 = dimorphic/slightly dimorphic	1 = not dimorphic	
	14. False vein	0 = present	1 = not present	
Sori	15. Location	0 = forking point	1 = terminal	2 = bending point
Indusium	16. Structure	0 = scaly	1 = not scaly	
	17. Attachment	0 = base only	1 = base and part of side	2 = base and along side
	18. Shape	0 = Semi to nearly circular	1 = pouched shape or square or nearly square	2 = reniform

Table 3.2: Morphological data matric

Taxa	**[00000000011111111111]** **[12345678901234567890]**
Leucostegia pallida	11010211011111110100
L. immersa	01010211011111110000
Oleandra colubrina	11010000000001130021
Scyphularia triphylla	11111010100011012100
*Davallia denticulata*_L	11010001101010012100
*D. denticulata*_ NS	11010001101010012100
*D. denticulata*_R	11010001101010012100
*D. divaricata*_F	11010101111001012100
*D. divaricata*_L1	11010101111001012100
*D. divarivata*_L2	11010101111001012100
*D. divaricata*_F2	11010101111001012100
*D. divaricata*_F3	11010101111001012100
*D. divaricata*_L3	11010101111001012100
D. dimorpha	11010111111101012100
*D. solida*_J1	11011011101011012100
*D. solida*_P	11011011101011012100
*D. solida*_J2	11011011101011012100
D. trichomanoides var. *lorrainii*_J	11001010111110012100
D. trichomanoides var. *lorrainii*_F	11001010111110012100
D. trichomanoides var. *trichomanoides*	11000000111110012120
D. corniculata	10000011111010011020
Araiostegia hymenophylloides	11010021111111200000
*Humata vestita*_L	10000011100100010020
H. vestita	10000011100100010020
H. parvula	10000011100001010000
*H. angustata*_F	10000001100101010000
*H. angustata*_K	10000001100101010000
H. pectinata	10000011100011010020
H. repens	10000011100011010000
*H. repens*_J1	10000011100011010000
*H. repens*_J2	10000011100011010000
*H. repens*_L	10000011100011010000
*H. repens*_F1	10000011100011010000
*H. repens*_F2	10000011100011010000
H. heterophylla	10000010100001010000

3.3.2. Morphological Phylogeny

A heuristic search was performed with 36 taxa using *Oleandra colubrina* as outgroup taxon. The analysis resulted in 117 most parsimonius trees. The length of the trees was 43 steps (Figure 3.6). Sixteen of eighteen characters were parsimony informative and two were parsimony uninformative. The Consistency Index (CI) was 0.51, Retention Index (RI) 0.86 and the Rescaled Consistency Index (RC) was 0.44. The bootstrap value ranged between 53% and 81%.

The strict consensus tree (Figure 3.7) of the morphological analysis was not well resolved with most of the ingroup taxa showing a large polytomy and few nested. All *Humata* species were nested together to form a separate clade with *Davallia* species as sister clade.

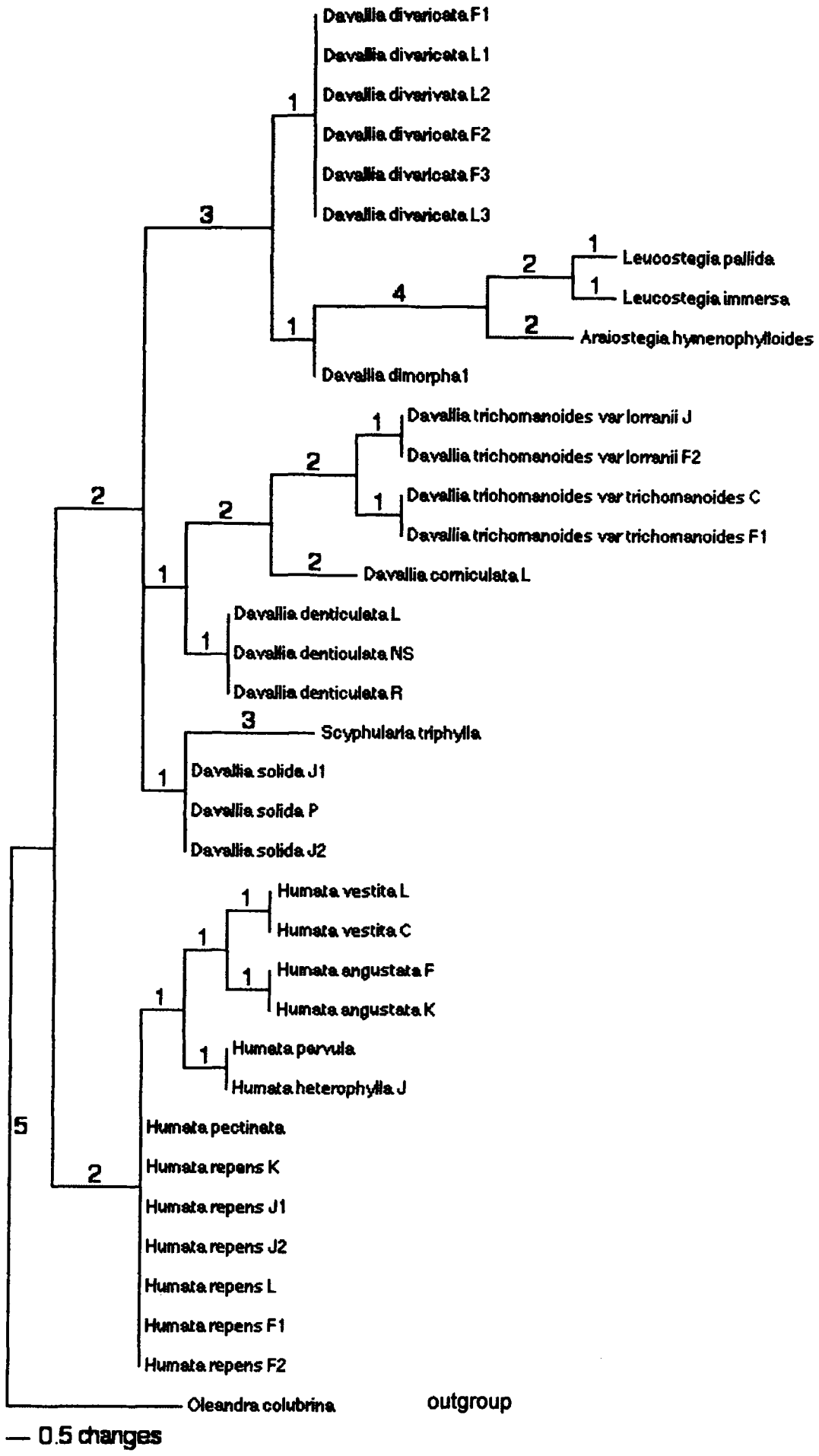


Figure 3.6: Tree one of most parsimonious trees (tree length = 43, CI = 0.51; RI = 0.86; RC = 0.44) based on 17 morphological characters. Numbers above each line shows branch length.

Fig. 3.7: Strict consensus tree of most parsimonious trees (tree length = 43, CI = 0.51; RI = 0.86; RC = 0.44) based on 17 morphological characters. Numbers above branches indicate bootstrap support.

3.4 **Discussion**

The application of morphological data in phylogenetic study is challenging. There are also no standard methods in coding and scoring the morphological characters for phylogenetic analysis. Qualitative data in morphological phylogenetic studies further contribute to these difficulties. As a result, the phylogenetic tree constructed depends on how morphological characters were chosen and coded by the researcher. Therefore morphological results have to be interpreted with caution as the way of coding characters can be subjective. However the opposite occurs when molecular data are used in phylogenetic study as various software packages have been developed to facilitate characters/regions to be used.

There are many factors which influence morphological phylogenetic study for instance low numbers of characters especially in pteridophytes where most characters used are gathered from the sporophyte. This has lead to a higher incidence of homoplasy. Suggestions have been made to increase the number of characters or to use molecular data in conjunction with morphological data to decrease homoplasy.

The use of morphological characters in phylogenetic analysis remains controversial. This is particularly so if morphological and molecular data were both combined. Scotland et al. (2003) stated that combination of morphological characters with molecular data in phylogenetic analysis will create more

ambiguity and homoplasy. However Wiens (2004) disagreed; he reasoned that phylogenetic analysis using morphological data can help to establish relationships of most fossil taxa and living taxa. In addition, he also suggested that when interpreting molecular data, morphological phylogenetics can be used to complement data and adds accuracy.

Morphological phylogeny based on parsimony search

In general the branch support values in the tree based on morphological characters were very low. This is common in morphological phylogenetic studies because of low number of characters. The bootstrap values will increase the higher the numbers of characters (Bremer et al., 1999). The same phenomenon exists in the present study, none of the clade was well supported (Figure 3.7). This is most likely a result of low numbers of characters used in this study (eightteen characters). Although the clades in the phylogenetic tree were not well supported, it still provides information on the evolution of characters studied in Davalliaceae.

Chapter Four

Chromosome counts

4.1 Introduction

Chromosomes provide a type of comparative data that is useful for addressing a variety of taxonomic and evolutionary questions (Moore, 1978; Grant, 1981). The chromosomes can determine many of the process involved in evolutionary change or can act as markers of them but they do not do so equally in all groups or in all situations (Moore, 1978). The information from chromosomes generally can be divided into three groups namely chromosome number, chromosome structure and chromosome behaviour (Stace, 1989; Singh, 2004).

Chromosome number is one of the most widely used cytological characters in taxonomy. This is because in vascular plants, there is great diversity of chromosome numbers (e.g. the lowest basic chromosome number (x = 2) in *Haplopappus gracilis* (Asteraceae) and the highest (x = 132 and x = 630) in *Poa littoroa* (Poaceae) and *Ophioglossum reticulatum* (Ophioglossaceae) respectively (Lovis, 1977). In addition, chromosome number frequently correlates with taxonomic groupings. It often demonstrates general stability and constancy within populations, species and genera. The classification of many families has been aided or substantiated by information on chromosome number and morphology.

Although cytological data have been used since 19th century in flowering plants, in ferns the use of chromosome data started in 1950 with the publication of "Problems of cytology and evolution in the pteridophytes" by Manton (1950). She introduced the squash technique which stimulated research on a worldwide basis. Many fern floras have been sampled cytologically since then, including Britain (Manton, 1950); Madeira (Manton, 1950; Manton et al., 1986); Jamaica (Walker, 1966) and Malaya (Manton, 1968).

Most of the studies showed a uniform base number for a genus or family, or ranges around a given number. Homosporous ferns generally have a high basic chromosome number, for instance the genus *Osmunda* has x = 22, *Pteris* 29, *Asplenium* 36, *Dryopteris* 41, *Botrychium* 45 and *Pteridium* 52. However a low number (x = 11) has been reported in the filmy fern *Hymenophyllum peltatum*. In contrast, heterosporous ferns have low base numbers (*Marsilea,* x = 10, 13, or 19; *Salvinia,* x = 9; *Azolla,* x = 22).

The addition or loss of one or two whole chromosomes is referred as aneuploidy. Examples of aneuploidy are *Lomariopsis* (Lomariopsidaceae) which has basic chromosome numbers ranging from 16 to 41 and *Thelypteris* s.l (Thelypteridaceae) with a range of 27 to 36. Variation in the basic chromosome number suggests that the "genus" concerned may be unnatural (Lovis, 1977).

Polyploidy is defined as having multiple sets of chromosomes, a common phenomenon among ferns. A single genus may have diploid (two times the base number), tetraploid (four times) and hexaploid (six times) chromosomes counts as reported in *Adiantum* and *Asplenium*. Species with both diploid and tetraploid forms are common, especially among widespread, abundant ferns. There are two major forms of polyploid: autopolyploidy (union of three or more chromosome sets from same species) and allopolyploidy (union of two or more different genomes or species). Autopolyploidy is less common in plants as chromosome duplication will result in a plant with four homologous sets of chromosomes; the result may be failure of regular chromosome pairing during meiosis and subsequent sterility. In contrast, allopolyploidy usually arises by hybridization followed by chromosome duplication, establishing a tetraploid with two sets of homologous chromosome pairs, giving normal pairing at meiosis and subsequent fertility (Judd et al., 1999).

In early cytological studies based on European flowering plants, it was suggested that the percentages of polyploidy tend to increase with increasing latitude. The percentage was relatively low in southern Europe and the Mediterranean basin and rising to over 85% in the extreme northern part. As temperature is one of the most obvious environmental features that varies with latitude, the suggestion was advanced that polyploidy was enhanced by cold, and the same principal was applied to often higher percentages of polyploidy founded on some mountain ranges (Walker, 1979). However Manton concluded that occurrence of polyploidy is correlated with variable climatic

changes, history of geographical disturbances, moderately high latitude and regions with rich flora and not dependant on a single ecological factor. Malaysia, despite its tropical climate, has only about 42% of polyploidy as the flora was less disturbed by climate changes. This can be compared with the Madeira (25%), Himalaya (35%) and Japan (45%).

Chromosome counts for Malaysian ferns first began with a list of chromosome numbers for a total of 100 species (15%) in 48 genera by Manton. The list was published as an appendix in Flora of Malaya volume 2 (ferns) by Holttum (1968). This work has stimulated study on Malaysian ferns, namely Walker and Jermy (1982), Bidin (1984; 1995), Bidin and Walker (1994) and Bidin and Go (1995). However with the utilization of molecular studies in fern, cytological study has been less popular. For Malaysian ferns, there has been no new cytological data in the last ten years. Only 138 taxa (16%) have been studied between 1955 and 1995 (Bidin, 2000). The present study contributes additional information on chromosome numbers of Davalliaceae in Peninsular Malaysia.

4.2 **Materials and Methods**

The materials used to make cytological squash preparations were obtained from a selection of young sporangia, light green in colour, located on the apical part of fronds and derived from two sources:

i. Fixings of fresh sporangial material during field collection in June 2005 and,

ii. Fixings of sporangia and root tips/croziers from plants which were brought from the field and cultivated in the glasshouse at the Royal Botanic Garden Edinburgh (RBGE).

In the field, all sporangial materials were fixed for 24 hours in one part glacial acetic acid to three parts absolute alcohol (Farmers solution). After 24 hours fixation, the sporangia were transferred to 70% alcohol and stored in refrigerator or freezer when available. At RBGE, the materials were fixed in acetic acid and alcohol and stored immediately in the freezer at -20 ^{0}C. After 24 hours, the fixed materials were transferred to 70% alcohol and again stored in the freezer at -20 ^{0}C.

For meiotic observation, Manton's (1950) squash technique was replicated. The fertile frond was removed from the fixing bottle with a forceps and placed on a clean slide in a drop of aceto-carmine solution. Groups of sporangia were separated from the indusium with a needle. A coverslip was then placed over the sporangia and the slide was gently heated over a spirit lamp until the aceto-carmine began to boil. The slide was then immediately covered with filter papers and squashed as hard as possible. Good preparations were made permanent by the alcohol exchange method without removal of coverslips as suggested by Bradley (1948).

For some samples it proved difficult to obtain suitable sporangia material for meiotic preparation (e.g. *Humata pectinata* and *Scyphularia triphylla*) and so root tips and young croziers were sampled to obtain mitotic preparations. Root tips or croziers were pre-treated prior to fixation to inhibit spindle formation. The samples were washed a few times with tap water and once with distilled water before pre-treatment in para dicholorobenzene (PDB); this increases the proportion of cells at metaphase by inhibiting the formation of the spindle. The materials were left for three to four hours in the dark at room temperature to enable cell division to continue in the pre-treatment solution. After pre-treatment, the samples were rinsed with tap water to remove PDB, then transferred to Farmer's solution and stored in the freezer.

Prior to squashing, the mitotic samples were place in 5N HCL at 60^0C for 30 minutes before staining in Feulgen solution and softening with enzyme (4% pectinase and cellulose). The staining with Feulgen takes two hours in dark conditions while softening takes another 30 minutes in the pre-heated water bath. The stained material was then placed on a slide and squashed in 45% acetic acid or aceto-carmine. Good preparations were made permanent using the alcohol exchange method. The fully dried permanent slides were then observed again under Axiophot Zeiss light microscope and photographs were taken using AxioVision in bright field with 60X or 100X objective/magnification. Chromosomes counting was done at least 3 times on same cell of the photographs (Figure 4.1).

4.3 **Result**

Chromosome counts were obtained from meiotic or mitotic materials of samples collected and results are summarized in Table 4.1.

1. ***Araiostegia hymenophylloides*** **(Blume) Kuhn**

This species is distributed in Negeri Sembilan, Pahang, Penang and Perak. The material was collected only from Bukit Larut, Perak and has a diploid meiotic chromosome number of n = 40, i.e. 40 pairs of chromosomes (2n = 80) (Figure 4.1a). This is the first cytological report of the species in Malaysia. The result is consistent with previous reports on this species from Ceylon (Sri Lanka) by Manton and Sledge (1954).

2. ***Davallia corniculata*** **T. Moore**

This species is distributed in Pahang, Perak, Selangor and Terengganu. A meiotic chromosome count was obtained from a plant in Cameron Highlands, Pahang. The chromosome number is diploid with n = 40 (Figure 4.1b). Manton (1955) has reported the same number for this species from Malaya (the exact locality in Malaya unknown).

3. ***Davallia divaricata*** **Blume**

This species is distributed in Kedah, Langkawi Islands, Pahang, Perak and Tioman Island. The sample was collected in Fraser Hill, Pahang. The

meiotic material showed n = 40 (Figure 4.1c) and it is a new cytological report of the species in Malaysia.

4. ***Davallia trichomanoides*** **Blume var.** ***lorrainii*** **(Hance) Holttum**

This species is distributed in Kedah, Negeri Sembilan, Pahang, Penang and Selangor. The meiotic chromosome number n = 40 was obtained from material collected in Cameron Highlands, Pahang (Figure 4.1d). This is the first cytological report of the species in Malaysia.

5. ***Davallia trichomanoides*** **Blume var.** ***trichomanoides***

This species is only reported in Pahang. The meiotic chromosome number n = 40 was obtained from material collected in Cameron Highlands, Pahang (Figure 4.1e). This is the first cytological report of the species in Malaysian flora.

6. ***Scyphularia triphylla*** **Hook.**

This species is distributed in Johore and Terengganu. The meiotic material was collected in Gunung Pulai, Johore and showed a diploid chromosome number n = 40 (Figure 4.1f). This is the first report for this species in study area.

7. ***Humata angustata*** **Wall. ex Hook. & Grev.**

This species is distributed in Johore, Kedah, Kelantan, Melaka, Negeri Sembilan, Pahang, Penang, Perak, Selangor and Terengganu. The meiotic material was collected in Fraser Hill, Pahang and showed a diploid chromosome number, n = 40 chromosomes (Figure 4.1g). This is the first cytological report of the species in Malaysia.

8. ***Humata pectinata*** **Sm.**

This species is distributed in Johore, Kelantan, Langkawi Islands, Melaka, Pahang and Terengganu. The mitotic material was collected from Gunung Jerai, Kedah (new site), and possessed 2n = 80 chromosomes (Figure 4.1h). This is the first cytological report of the species in Malaysia.

9. ***Humata repens*** **(L.) Kuhn**

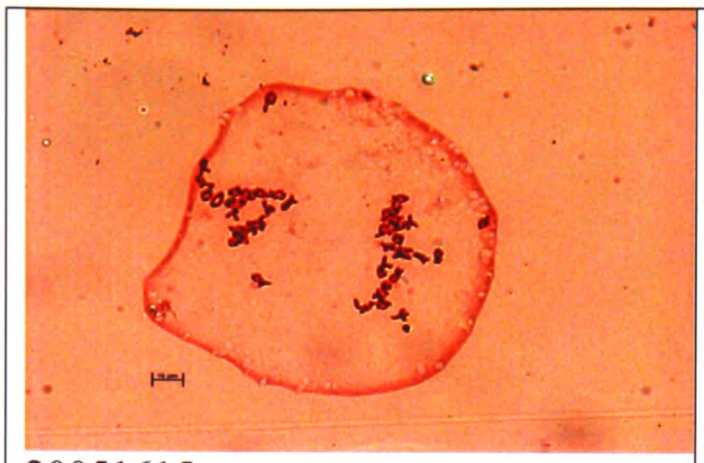
This is a very common species and distributed in Johore, Kedah, Kelantan, Pahang, Penang, Perak and Selangor. The chromosome count of the species obtained from Gunung Jerai, Kedah, showed a diploid chromosome number n = 40 (Figure 4.1i). The Ceylon sample has been reported as having 2n = 120 (Manton and Sledge, 1954). This is the first cytological report of the species in Malaysia.

10. ***Humata vestita*** **(Blume) T. Moore**

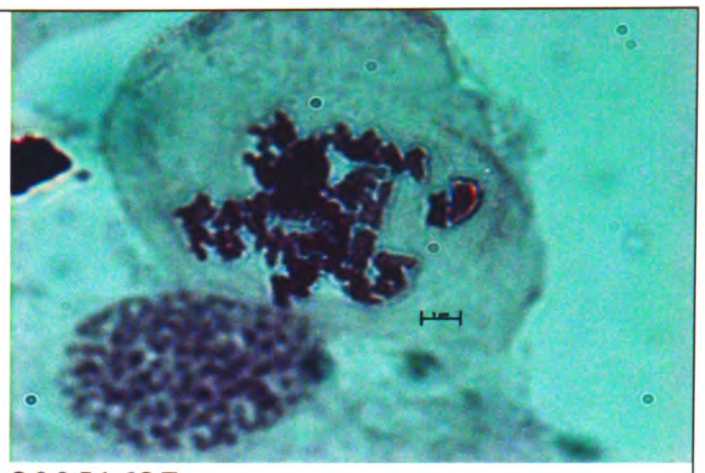
This species is reported in Pahang as a terrestrial plant. The meiotic material obtained from Cameron Highlands, Pahang, showed a different chromosome number from other species of *Humata* of n = 120, a hexaploid (Figure 4.1j). This is the first cytological report of the species in Malaysia.

11. ***Leucostegia immersa*** **C. Presl**

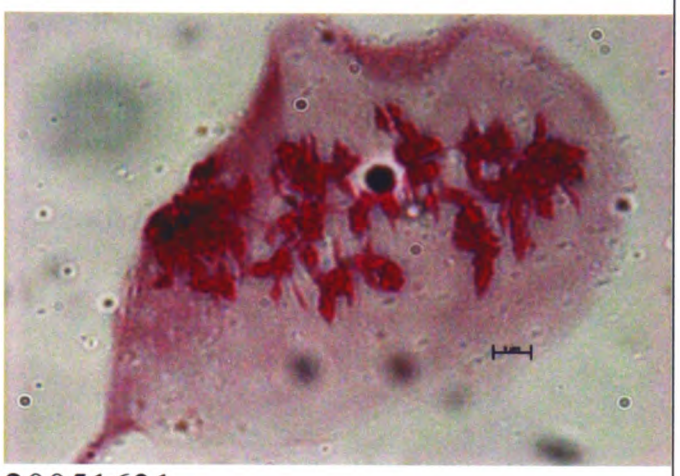
This species is reported in Pahang as an epiphyte. The meiotic material obtained from Cameron Highlands, Pahang, showed telophase stage II with ca 160 chromatids (Figure 4.1k), equivalent to a meiotic count at metaphase 1 of n = ca 40. However chromosome counts from India and Taiwan showed n = 41 (Tryon and Lugardon, 1991). This is the first cytological report of the species in Malaysia.



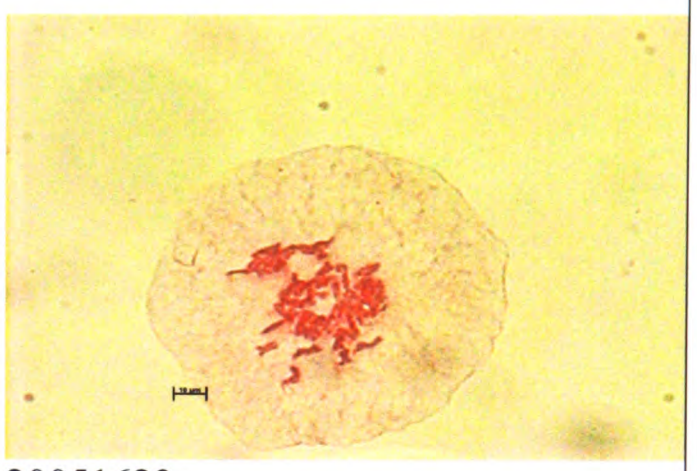
20051615
a. *Araiostegia hymenophylloides*



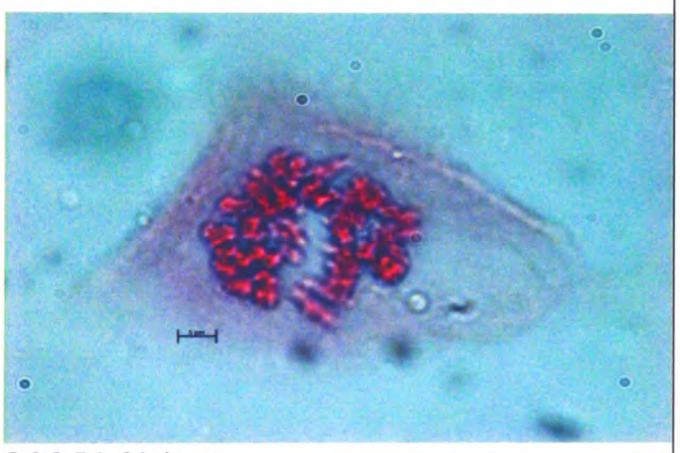
20051627
b. *Davallia corniculata*



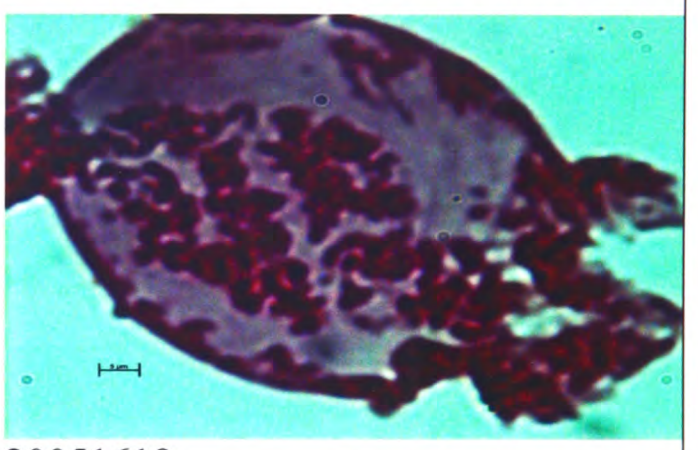
20051631
c. *Davallia divaricata*



20051623
d. *Davallia trichomanoides* var. *lorrainii*

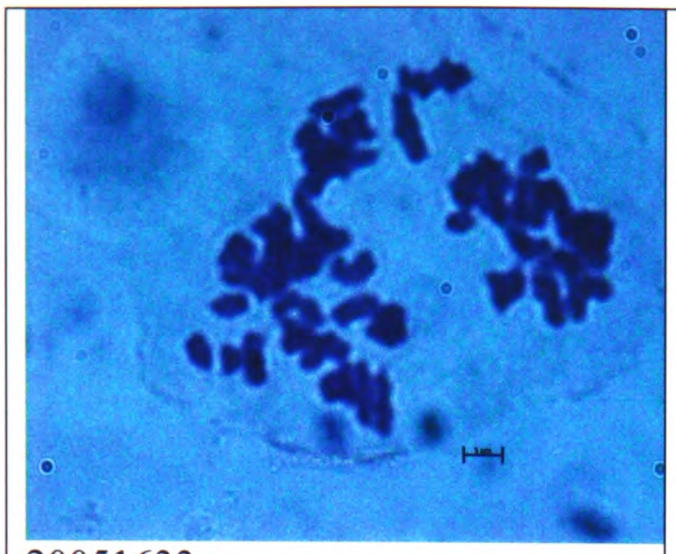


20051614
e. *Davallia trichomanoides* var. *trichomanoides*

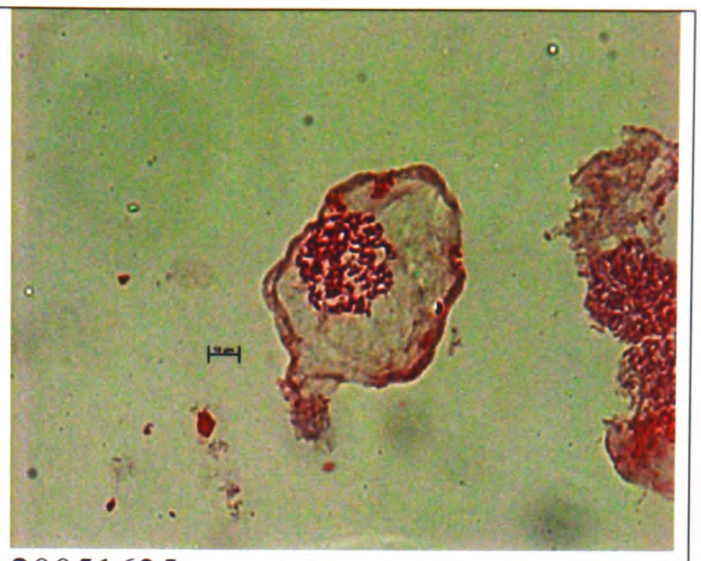


20051619
f. *Syphularia triphylla*

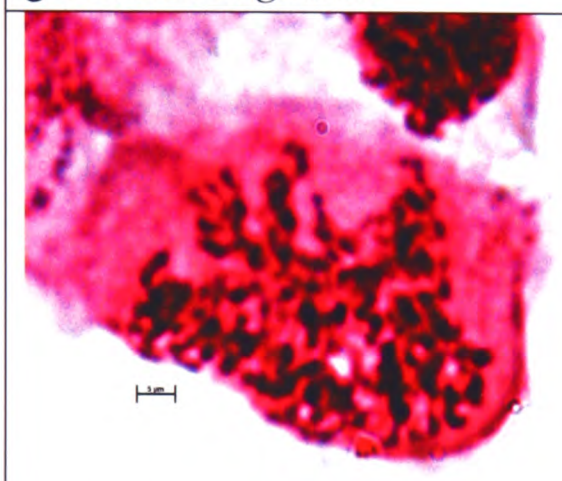
Figure 4.1. Chromosome of Davallioid species with accessions or specimen number.



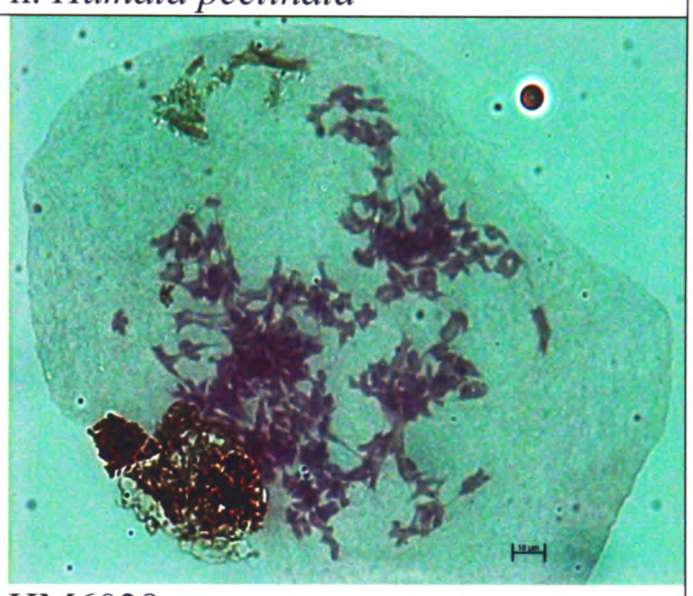
20051633
g. *Humata angustata*



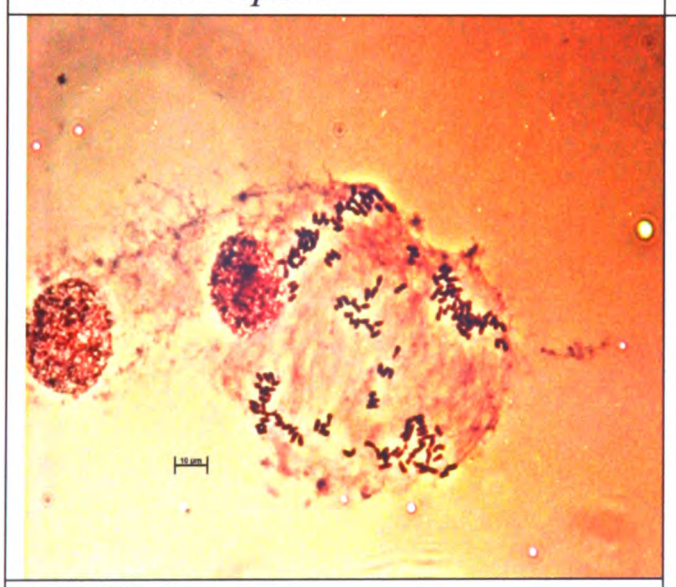
20051635
h. *Humata pectinata*



HM6019
i. *Humata repens*



HM6039
j. *Humata vestita*



20051617
k. *Leucostegia immersa*

Figure 4.1. (Continue)

4.4 Discussion

4.4.1 Timing of material collection and fixing

Good cytological slide preparation depends on the time of fixation. In this study most results were obtained from materials which were fixed around midday (11.30 am – 12.30 pm) in the glasshouse. The midday period is known to be at the peak of cell division in many plants and thus will have the highest numbers of metaphase when fixed for cytological observation. The time recommended proves to be generally satisfactory in many plant families (Jong, 1997). In Malaysia, a good fixing time for cytological study is before sun rise between 7.00 - 7.30 am (R. Jaman, pers. comm.). This time is not practical in the field, so plants were kept in a glass with water before spore mother cells were collected and fixed the next morning.

4.4.2 Chromosome number

In this study, new cytological data were obtained from thirteen species of Davalliaceae from Peninsular Malaysia. The chromosome numbers obtained from spore mother cells, croziers and roots showed that the majority of species are diploid, with ca. 40 bivalents at meiosis or 2n = 80 from mitotic preparations.

Chromosome counts of four species previously studied by Manton (*D. denticulata*, *D. corniculata*, *H. heterophylla* and *H. parvula*) showed that all the 4 species are diploid with n = 40 or 2n = 80 (Table 4.1). Only one species

(*D. corniculata*) had been studied previously from Peninsular Malaysia and the earlier count was confirmed.

Araiostegia hymenophylloides has a chromosome number of n = 40. This is consistent with finding made by Manton and Sledge (1954) on a sample from Ceylon. However Singh and Roy (1988) reported a chromosome number for a species of *Araiostegia* from Himalaya as n = 41 (aneuploidy), and suggested that n = 40 is more primitive than n = 41. Aneuploidy is uncommon in ferns. Panigrahi and Patnaik (1964 in Singh and Roy, 1988) have commented that the aneuploid, a method of reduction or increase in the base numbers have played a major role in evolution and adaptation of fern to epiphytic habitats. In Malaysian ferns, the occurrence of aneuploidy is noted only in *Adiantum polyphyllum* (Bidin, 2000).

It is probable that the basic chromosome number 40 is characteristic for Davalliaceae. All species examined are diploid except *Humata vestita*, which is hexaploid (polyploidy) with n = ca. 120. No previous data on chromosome numbers for *H. vestita* was available for comparison. *H. vestita* is distinct from *H. repens* in chromosome number, despite being treated as a synonym of *H. repens* by Nooteboom (1998).

The spore size of hexaploid *H. vestita* is larger (>80μm) than the other (diploid) species in this group. This phenomena is common in polyploidy as chromosome doubling leads to increase in nucleus and cell size. Ferns spores

are frequently larger in polyploids compare with diploid parents (Moran, 1982). The complete result of chromosome counts of Davalliaceae in Peninsular Malaysia are presented in table 4.1.

Polyploidy is an important speciation mechanism in plants. The number of species that have arisen by polyploidy is enormous, making up at least 30% of angiosperms (Stebbins, 1950) and 45% of pteridophytes (Vida, 1972). The percentage of polyploidy in Malaysian ferns has been reported to be low, only at 42% (Bidin, 2000) compare with 60% in Ceylon ferns (Manton and Sledge, 1954). This could be attributed to the locality, habit of the species and very few taxa that have been analysed cytologically. Manton (1969 in Walker, 1979) suggested that part of the land mass represented by Malaysia, together with the Mediterranean basin which is also rich in diploids, may support a Tertiary flora which has been less disturbed by climatic changes that seem to have stimulated polyploidy elsewhere.

Table 4.1: List of taxa and chromosome number of Malaysian Davalliaceae

Species	Chromosome number		Ploidy	Data source
	meiosis	mitosis		
Araiostegia hymenophylloides	40 bivalents		2x	Present study
Davallia corniculata	40 bivalents		2x	Present study; Manton, 1955
Davallia denticulata	40 bivalents		2x	Manton, 1955
Davallia dimorpha	40 bivalents		2x	Present study
Davallia divaricata	40 bivalents		2x	Present study
Davallia solida	40 bivalents		2x	Present study
Davallia trichomanoides var. ***lorrainii***	40 bivalents		2x	Present study
Davallia trichomanoides var. ***trichomanoides***	40 bivalents		2x	Present study
Scyphularia triphylla		2n =80	2x	Present study
Humata angustata	40 bivalents		2x	Present study
Humata heterophylla	40 bivalents		2x	Manton, 1955
Humata pectinata	40 bivalents		2x	Present study
Humata repens	40 bivalents		2x	Present study
Humata parvula		2n = 80	2x	Manton, 1955
Humata vestita	ca. 120		6x	Present study
Leucostegia immersa	ca. 160 * chromatids		2x	Present study

* at telophase II

Chapter Five

Molecular study

5.1 Introduction

5.1.1 Molecular data and fern systematics

The development of techniques in molecular sequence amplification and phylogenetic analysis has given pteridologists the opportunity to test fern classifications that were previously based on morphology, anatomy or cytological characters. One of the first study using nucleic acid data for fern systematics was that of Stein (1985) for *Osmunda*. However, it was not until the study of Hasebe and Iwatsuki (1990), which utilized molecular data for inferring relationships among species within genera and among closely related genera, that the interest began. It was further escalated after a symposium in 1994, organized by the American Fern Society and Pteridological Section of Botanical Society of America, on the application of molecular data for studying ferns. By the time of this symposium, most pteridologists were already using molecular DNA studies to assist in the study of relationships among ferns.

Systematists use molecular data from three organelles in plants: chloroplast, mitochondrion and the nucleus. Among these organelles, the chloroplast genome is the smallest (135 - 160 kbp), followed by the mitochondrion (200 –

2500 kbp) and the nuclear genomes ($1.1 \times 10^6 - 110 \times 10^9$ kbp) (Judd et al., 1999)

In plants the chloroplast genome is more frequently studied than the other two genomes for phylogenetic investigation because chloroplast DNA (cpDNA) can easily be isolated, analyzed, and evolution rate is relatively slow. Among the frequently used chloroplast regions for ferns are *rbcL, trnL-F, matK* and *rps4-trnS.*

RbcL data has been successfully employed at generic level in phylogenetic study of ferns (e.g. Hasebe et al., 1995; Pryer et al., 1995). The *rbcL* sequence data were shown to be useful for generic and specific relationships among the heterosporous ferns (Pryer, 1999), the chelanthoid ferns (Gastony and Rollo, 1995) and within the genus *Polystichum* (Little and Barrington, 2003). However, Ebihara et al. (2002) found insufficient variation for phylogenetic resolution in the *rbcL* sequences for the genus *Hymenophyllum* s.l., so they included sequences from the *accD* + *rbcL* IGS (intergenic spacer) chloroplast regions to provide a more resolved and well supported phylogeny. The *trnL-F* spacer region has also been used increasingly, for instance in Ophioglossaceae (Hauk et al., 1996; 2003), Schizaeaceae (Skog et al., 2002), and *Asplenium* (Pintér et al., 2002). This intergenic spacer region provided informative phylogenies for these groups, and was valuable for differentiating species within them. Cranfill (2000) noted the usefulness of *rps4* and *rps4-trnS* IGS for land plant phylogenies, and Smith and Cranfill (2002) obtained informative

phylogenies for Thelypteridaceae using *rps4* and *rps4-trnS* IGS sequences. Recently Schneider et al. (2004a) found sequences of *rbcL, trnL-F* and *rps4-trnS* to be informative within the genus *Asplenium*.

5.1.2 The *rbcL, trnL-F* and *rps4-trnS* chloroplast DNA regions

The sequence data presented in this thesis are derived from the *rbcL* gene, *trnL-F* region and *rps4* + *rps4-trnS* IGS regions from the chloroplast. The *rbcL* gene is known as a protein coding region as it codes for RuBisCo, which is an enzyme used in photosynthesis. As a coding region, the *rbcL* sequence is composed of a series of codons which code individual amino acid. The codon position will show variable rates of variation and functional constrains within the codon. The length of this region is ca. 1400bp.

The *trnL-F* region is one of the non coding regions and consists of the *trnL* (UAA) 5' exon and the intergenic spacer between *trnL* (UAA) 3' exon and *trnF* (GAA) gene (Taberlet et al., 1991). The evolutionary rate of this region compared with *rbcL* rates varies, from similar rates to three times faster: the evolutionary rate of *trnL-F* is almost the same as the *rbcL* rate in Orchidaceae (Whitten et al., 1996), however in Ophioglossaceae, the rate is enhanced 3 to 5 times (Hauk et al., 1996). The length of this region is ca. 1000bp. According to Taberlet et al. (1991), this region is easily amplified with universal primers. However following discussion, the universal forward primer (C) was replaced with primer C for fern or "Cfern" (Trewick et al., 2002).

Rps4-trnS region encompasses the *rps4* gene (which encodes protein 4 of the small chloroplast ribosomal subunit) and an intergenic spacer. Nadot et al. (1994) pioneered the use of this region in their phylogenetic study of Poaceae. The length of this region is 1100 bp, enabling easy amplification.

5.1.3 **Molecular study of Davalliaceae**

A main aim of this chapter is to use phylogenetic analysis of various chloroplast markers to investigate the monophyly of genera and groups within the Davalliaceae, and the relationships of its species. The first application of molecular data in phylogenetic analysis of this family/group was by Tsutsumi and Kato (2005). Their study, based on five continuous chloroplast regions (*atpB, rbcL, accD, atpB-rbcL* spacer, and *rbcL-accD* spacer), indicated that none of the genera in the family was monophyletic (Figure 5.1). *Araiostegia* and *Davallia* were divided into two and three clades respectively, and *Humata* and *Scyphularia* were paraphyletic.

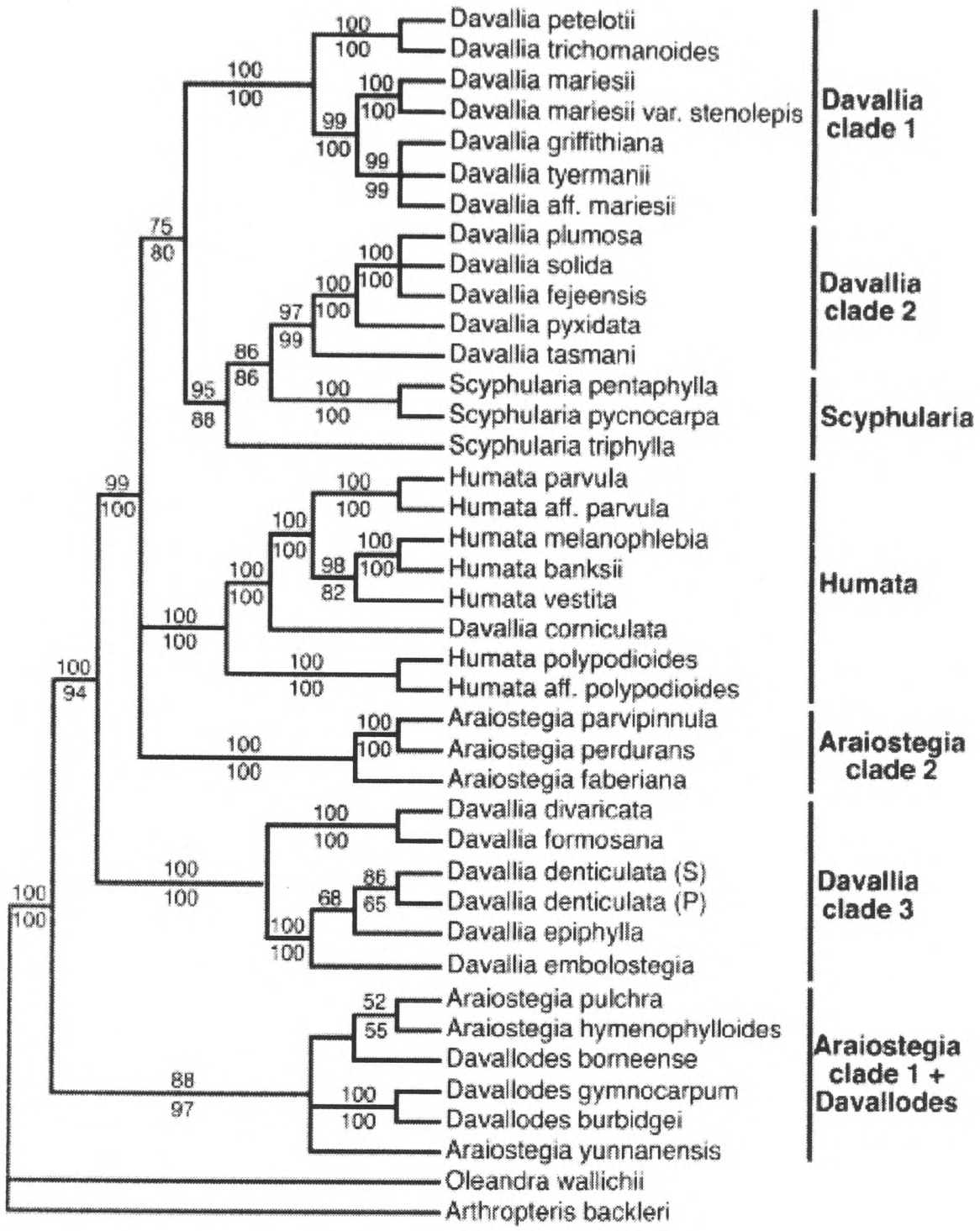


Figure 5.1 Strict consensus tree of four most parsimonious trees of Davalliaceae obtained by the MP method based on combined five regions sequences. Numbers above branches indicate bootstrap values (after Tsutsumi and Kato, 2005)

5.2 **Materials and Methods**

5.2.1 **Outgroup sampling**

Previous studies of fern phylogeny (e.g. Hasebe et al., 1995; Tsutsumi and Kato, 2005; Smith et al., 2006) have reported the genus *Oleandra* as a sister clade to davallioid ferns. In the present study, the outgroup consists of three species: *Oleandra, Arthropteris backleri* and *Leucostegia. Leucostegia* is classified as an outgroup because molecular data study indicated that it fall out side Davalliaceae (Tsutsumi and Kato, 2005).

5.2.2. **Ingroup sampling**

All species of Davalliaceae reported to occur in Peninsular Malaysia by the most recent studies (Parris and Latiff, 1997; Nooteboom, 1992, 1994, 1998) have been collected from the field for DNA extraction, except for *Humata parvula* and *Leucostegia pallida* which were not found in reported localities during field work. The DNA material for these two species was supplied by C. Tsutsumi. All species used were listed in appendix II

5.2.3 **DNA extraction, sequencing and aligment**

Procedures for extraction, amplification and sequencing for all three regions in this study were done according to RBGE molecular lab protocol vol I and II (Clark and Hollingsworth, 2006) and NHM molecular lab protocol (see Appendix III) The primers used are those published previously for the *rbcL* region (Gastony and Rollo, 1995), *trnL-F* region (Trewick et al., 2002;

Taberlet et al., 1991) and *rps4-trnS* region (Nadot et al., 1994 ; Smith and Cranfill, 2002) as listed in table 5.1.

Table 5.1 Primers used in PCR and sequencing reactions

Region	Primer	Direction	Sequence	Reference
trnL-F	Fern1	forward	5'-GGC AGC CCC CAR ATT CAG GGR AAC C-3'	Trewick et al. (2002)
	trnL-d	reverse	5'-GGG GAT AGA GGG ACT TGA AC-3'	Taberlet et al. (1991)
	trnL-e	forward	5'-GGT TCA AGT CCC TCT ATC CC-3'	Taberlet et al. (1991)
	trnL-f	reverse	5'-ATT TGA ACT GGT GAC ACG AG-3'	Taberlet et al. (1991)
Rps4-TrnS	rps4F	forward	5'-ATG TCM CGT TAY CGA GGR CCT CGT-3'	Nadot et al. (1994)
	trnS	reverse	5'- TAC CGA GGG TTC GAA TC-3'	Smith & Cranfill (2002)
rbcL	rbcL1F	forward	5'- ATG TCA CCA CAA ACA GAR ACT AAA GC-3'	Gastony and Rollo (1995)
	rbcL135IR	reverse	5'- CTT CAC AAG CAG CAG CTA GTT CAG GAC TCC-3'	Gastony and Rollo (1995)

All sequences were aligned manually using MacClade 4.0 (Maddison and Madisson, 2003). Alignment of *rbcL* data was guided by sequences of Tsutsumi and Kato (2005). Detailed alignment of the *trnL-F* and *rps4-trnS* sequences were checked using sequences from Genbank. Alignment of coding region *rbcL* was unambiguous, whereas alignment of the two non coding regions *trnL-F* and *rps4-trnS* included ambiguously aligned regions (All data included in attached CD).

5.2.4 **Data sets and ILD test**

There are three data sets: *rbcL*, *trnL-F*, *rps4-trnS*, each of which includes sequences for the same set of 36 taxa. Each data set was analyzed separately and together as a combined data set. All data sets were compared

using the incongruence length difference/ILD (Farris et al. 1994). The test was performed in PAUP ver. 4.0b (Swofford, 2002) using the Partition Homogeneity Test/PHT (see table 5.2). Frequency of unambiguous changes between states in both the non-coding region and each codon position in the coding region was estimated in order to approximate the degree of saturation present in each region. In PAUP, the beginnings and the ends of sequences in each region were determined (by using primer sequences). The beginnings and ends of sequenced regions, for which data was not available for all accessions, were excluded from further analyses. All areas of sequences where the alignment was ambiguous were excluded from the datasets, to avoid introducing artificial bias to the data. Fifteen and eight gaps were coded for the *trnL-F* and *rps4-trnS* matrix respectively using simple indel coding and the multistate gap region method (Simmons and Ochoterena, 2000; Simmons et al., 2001). No gaps were present in the *rbcL* matrix. In addition to these three data sets, the whole *rbcL* region data set of Davalliaceae (combining the present data set with previous data from Tsutsumi and Kato, 2005) were also compiled for analysis.

5.2.5 **Phylogenetic analysis**

For the phylogenetic reconstruction two different types of analyses were performed: Maximum Parsimony (MP) and Bayesian Analysis (BA). They were run on each of the three data sets with and without the inclusion of the gap matrix. The MP method is based on the assumption that evolution takes place by the simplest way (Crawford, 1990). In MP the most

parsimonious tree(s) is the tree that requires the minimum number of evolutionary changes to explain the data. But assumptions of minimal evolution are disputed by many cladists as this could underestimate the true number of changes. In MP it is assumed that a character state shared by two taxa is more likely to have been inherited from a common ancestor than that this character state has evolved more than once due to homoplasy (due to reversals, convergences, parallelisms) (Hall, 2004).

BA tries to find the most probable tree(s) given the sequence data and the model of evolution (Hall, 2004). BA considers that characters could have evolved more than once due to homoplasy and can allow for such changes. It compensates for these and is therefore considered to be superior in calculating distances compared with MP. It is a Likelihood method based on the concept of posterior probabilities. A tree search will look for the best set of trees and the same tree will often be considered several times during that process. In a BA it is required to include information about evolutionary processes by different evolutionary models.

5.2.5.1 **Parsimony analysis**

Reconstruction of phylogenetic relationships based on the Maximum Parsimony Method (Felsenstein, 1983) was done in PAUP version 4.0b10 (Swofford, 2002). Phylogenetic trees were generated as phylograms and strict consensus trees. Descriptive tree statistics were given by the consistency index (CI), retention index (RI) and rescaled consistency index (RC).

All trees were obtained from weighted, unordered characters. Multistate characters were interpreted as uncertain and gaps were treated as missing. Fifteen and eight indels were coded as additional characters for *trnL-F* and *rps4-trnS* respectively.

For character optimisation the option 'accelerated transformation (ACCTRAN)' was used, which favours reversals. Starting trees were obtained via stepwise addition. Heuristic searches were performed for all analyses with 10 000 RANDOM addition sequence replicates using TBR with MULTREES on, STEEPEST DESCENT off and branches collapse if minimum branch length is zero.

Branch support analyses were carried out using Bootstrap (Felsenstein, 1985) and Decay Indices (Bremer, 1988). Bootstrap values were calculated on 10,000 replicates with the same settings as above, except with only 1 RANDOM addition replicate. Decay Index was calculated with default settings in AutoDecay version 4.0 (Eriksson, 1999).

5.2.5.2 **Bayesian Analysis**

Parameters and the evolutionary model for all three regions were selected with the assistance of Modeltest version 3.07 (Posada and Crandall, 2001). The parameter and model selected on the Akaike Information Criterion (AIC) were used.

Partitioning of the data for model testing (Bayes likelihood) reflected natural structural differences in the three data parts. For the *rbcL* data, the first, second and third codon positions were modelled separately, whereas the *trnL-F* and *rps4-trnS* data were interpreted as whole partitions, with gaps treated as missing data. In total five partitions were classified: the first, second and third position of the *rbcL* gene, *trnL-F* and *rps4-trnS* gene. For the analyses, four independent Monte Carlo Markov Chains (MCMC) were run simultaneously for 1 million generations, starting with a random tree and with one tree saved every 100 generations. The analyses were also done with the inclusion of the gap matrix. The first 500 trees were discarded, and the burn-in for each run was determined by plotting the log likelihood of the cold chain versus the number of generations in Microsoft Excel.

5.3 Results

5.3.1 Sequence alignment

The combined length of all sequences was 3447 base pairs (bp). The *rbcL* sequence was the longest with 1330 bp following by *rps4-trns* and *trnL-F* with 1025 bp and 1092 bp respectively. An additional gap matrix was added to the *trnL-F* (15 characters) and *rps4-trnS* (8 characters) sequences. However these lengths were different from the numbers of characters included (Table 5.3) because of ambiguity at the beginning and end of sequences.

Table 5.2: Details of Incongruence Length Distance Test /ILD test/PHT

Parameters	*rbcL* vs *trnL-F*	*rbcL* vs *rps4-trnS*	*trnL-F* vs *rps4-trnS*	*rbcL* vs *trnL-F* vs *rps4-trnS*
Character included	2078	2118	1920	3278
Constant characters	1578	1671	1311	2421
Variable parsimony uninformative	135	126	177	247
Parsimony informative	365	321	432	610
P value	0.09	0.12	0.45	0.09

Table 5.3: Maximum parsimony data for *rbcL, trnL-F, rps4-trnS* and combined analyses

Parameter\Region	*rbcL*	*trnL-F*	*rps4-trnS*	Combined
Characters included	1138	940	980	3058
Number of parsimony informative characters	127	238	194	559
Number of variable uninformative characters	42	93	84	219
Number of constant characters	969	609	702	2280
Number of most parsimony tree (MPT)	1	1	1	1
Tree length (steps)	290	447	382	1077
CI	0.71	0.88	0.84	0.83
RI	0.86	0.94	0.93	0.92
RC	0.62	0.82	0.78	0.76
Transitions	130	118	213	-
Transversion	32	115	76	-

5.3.2 *rbcL* region

The aligment of *rbcL* sequence data contains 127 informative characters out of 1138 characters used. Table 5.3 summarises all the analysis information. Maximum parsimony (MP) analysis without ambiguity produced a single most parsimonious tree of 290 steps with CI = 0.71, RI = 0.86 and RC = 0.62. These values are relatively high indicating that the number of homoplastic characters was few.

In the Bayesian Analysis, the AIC selected model for *rbcl* region was based on each codon. The selected model for 1st codons was GTR+I+G [General time reversible; Tavaré (1986)], for the 2nd codons was JC+I [Jukes and Cantor, 1969] and TVM+G [transversional model] for the 3rd codons.

5.3.2.1 Tree topology

Maximum Parsimony

The strict consensus tree by MP analysis (Figure 5.2 and Figure 5.3) shows that the ingroup consists of six clades: the **Araiostegia clade**, four clades which together comprised the **Davallia clade** (i.e. **DCIa, DCIb, DCIIa and DCIIb)** and the **Humata clade**. The Araiostegia clade is highly supported, consisting of only *Araiostegia hymenophylloides* (bootstrap (bs) = 100%, decay value (d) = 2) which is a sister clade to all other clades. DCIa comprises *D. denticulata*, and is a sister clade to the DCIb (*D. dimorpha* and *D. divaricata*) clade (bs value for the sister relationship is high - 100%). DCIIa consists of *Scyphularia triphylla* and *D. solida* (bs = 93%, d = 1). DCIIb

consists of *D. trichomanoides* (both varieties; bs = 100% and d = 1). The Humata clade consists of *D. corniculata* as a sister clade to all *Humata* spp. (bs = 100%, d = 2).

Bayesian analysis

The topology of the Bayesian AIC majority rule consensus tree (Figure 5.4) shows slight differences from the MP tree. *A. hymenophylloides* and *D. denticulata* form a polytomy and sister clade to the rest of the Davallia and Humata clades. The posterior probability values (pp) range between 0.71-1.00.

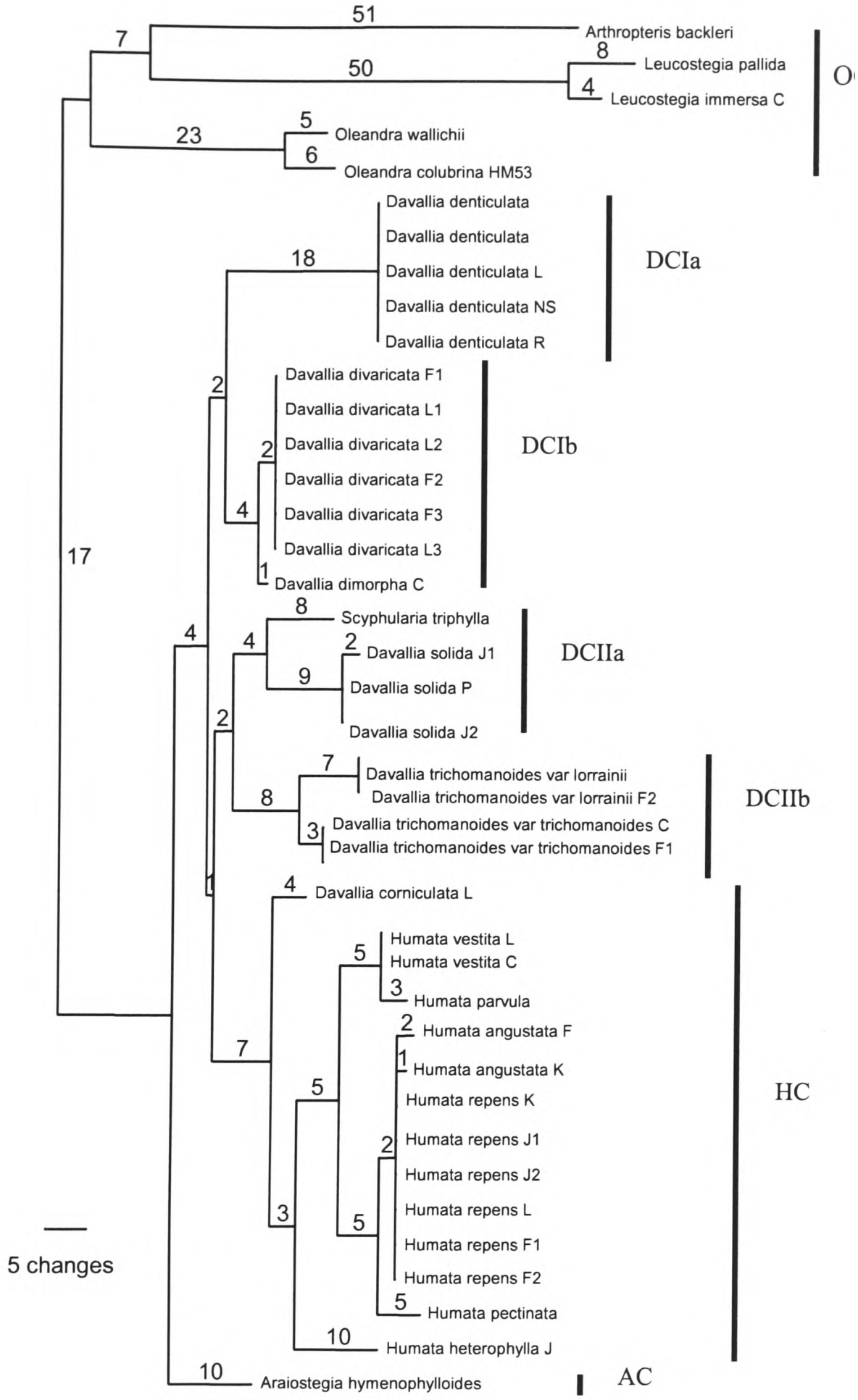


Figure 5.2: Phylogram of one most parsimony tree based on *rbcL* sequences with a heuristic search using weighted maximum parsimony analysis of *rbcL* including *Arthropteris backleri* and *D. denticulata* from Genbank (Tree length = 290 steps, CI = 0.71, RI = 0.86, RC = 0.62; OG = Outgroup, DC = Davallia clade, HC = Humata clade, AC = Araiostegia clade). The numbers above branches indicate branch length.

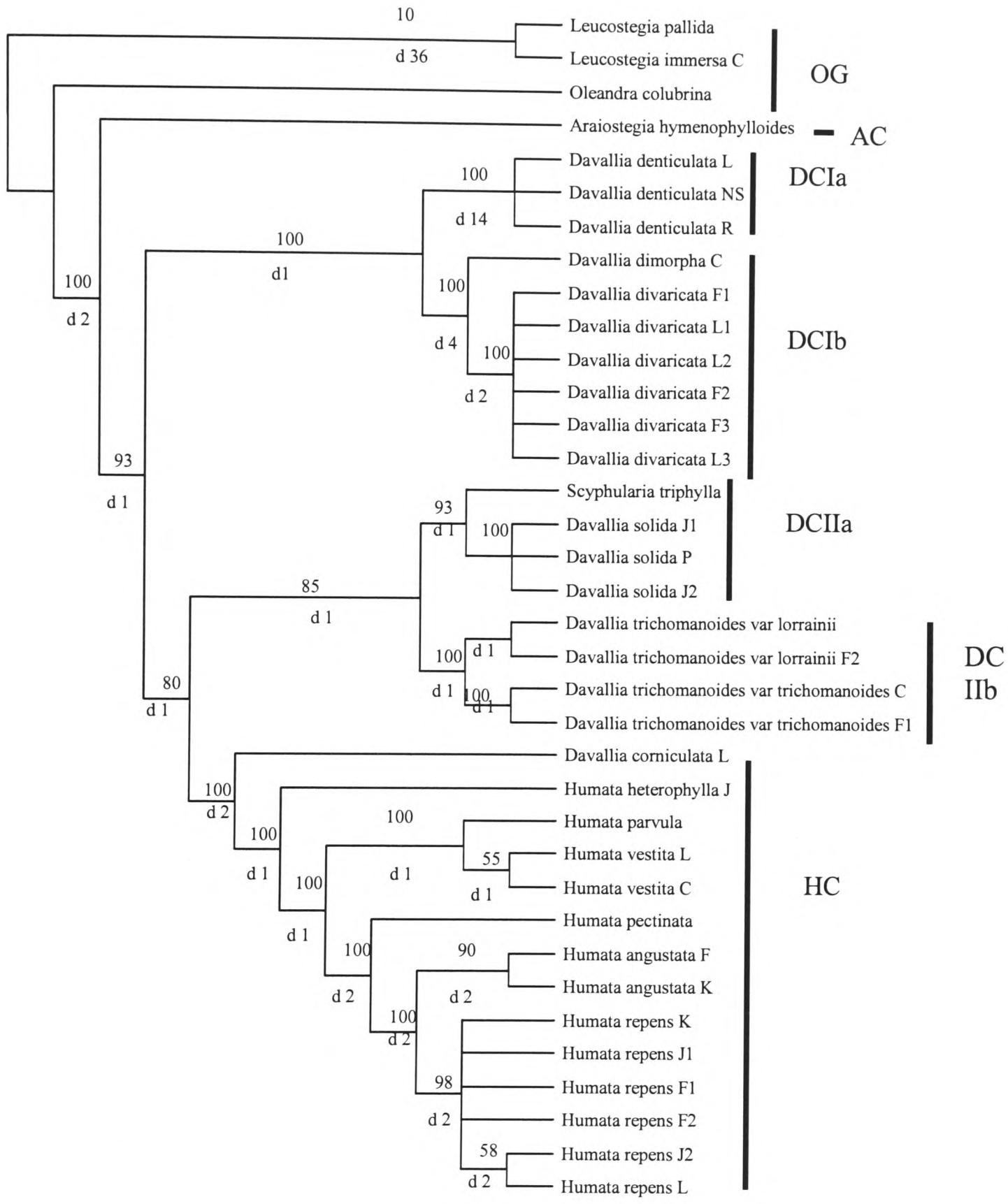


Figure 5.3: Strict consensus tree based on *rbcL* sequences with heuristic search using maximum parsimony analysis.(Tree length = 290; CI= 0.71; RI=0.86; RC=0.62). Numbers above branches indicate bootstrap support and numbers below branches indicate decay indices (d).

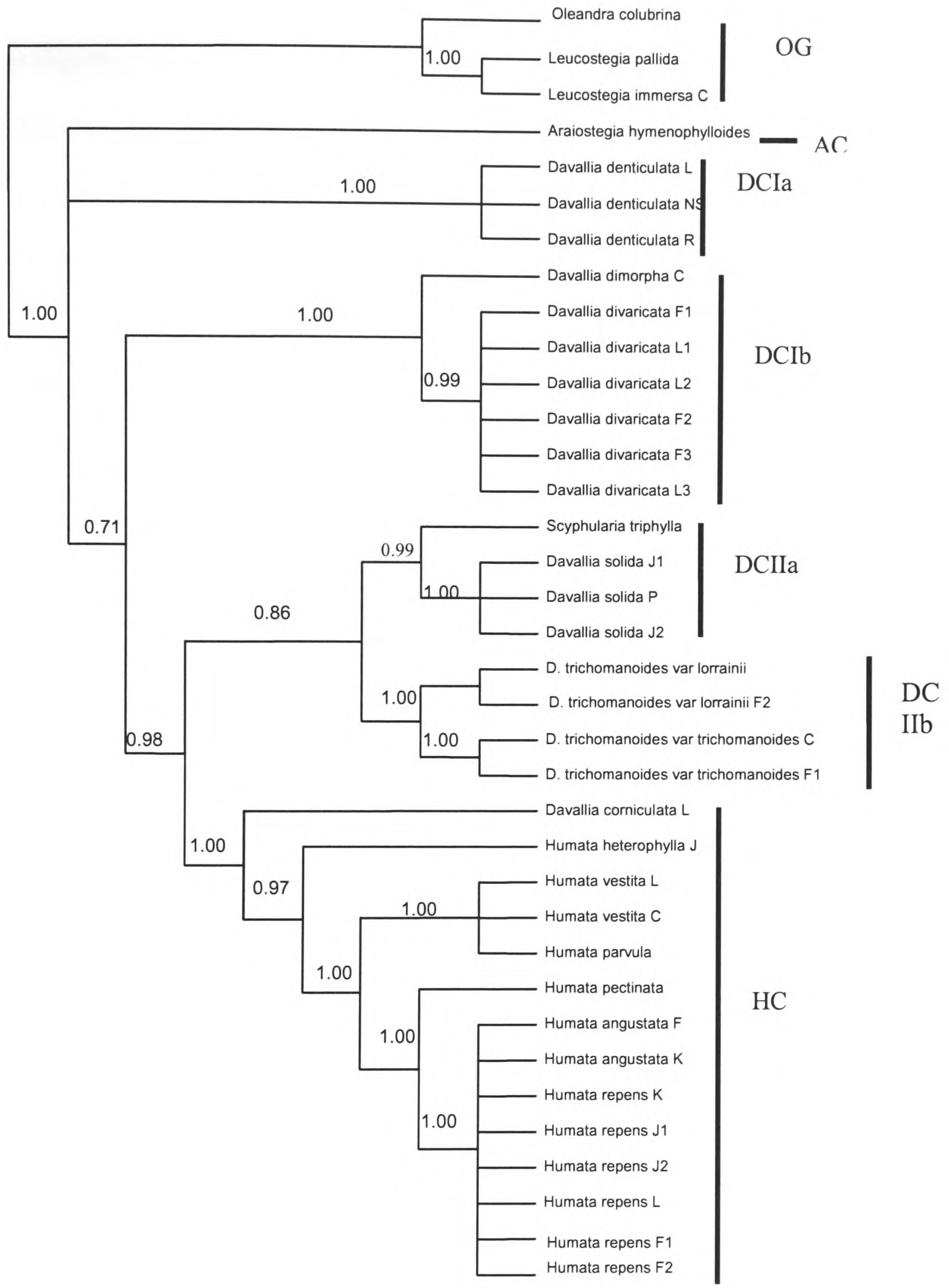


Figure 5.4: Bayesian majority rule consensus tree obtained from *rbcL* data matrix (36 taxa). Numbers above branches are estimated posterior probability values.

5.3.3 *trnL-F* region

The aligment of *trnL-F* sequence data contains 636 informative characters out of 924 characters used. Table 5.3 summarises all the analysis information. In the maximum parsimony (MP) analysis, the first analysis using data without ambiguity produced a single most parsimonious tree of 447 steps with CI = 0.88, RI = 0.94 and RC = 0.82. The second analysis with no ambiguity and no gap matrix produced a tree with 431 steps and CI = 0.88, RI = 0.94 and RC = 0.82, the same as with gap characters. The values are relatively high indicating that the number of homoplastic characters was low. The AIC selected model for *trnL-F* was K81 uf+1 [Two transversion-parameter model I unequal frequencies; Kimura (1981)].

5.3.3.1 Tree topology

Maximum Parsimony

The strict consensus tree by MP analysis (Figure 5.5 and 5.6) shows that the ingroup consists of six clades: the **Araiostegia clade**, four clade which together comprised the **Davallia clade (DCIa, DCIb, DCIIa and DCIIb)** and **Humata clade**. The **Araiostegia clade** is weakly supported, consisting only of *Araiostegia hymenophylloides* (bs < 50%, d = 41) which is a sister clade to all other clades, as in the *rbcL* MP tree. Davallia clade Ia consists of *D. denticulata* (bs = 100%, d = 13), Davallia clade Ib consists of *D. dimorpha* and *D. divaricata* (bs = 100%, d = 7), Davallia clade IIa consists of *Scyphularia triphylla* and *D. solida* (bs = 51%, d = 1), Davallia clade IIb consists of *D. trichomanoides* (both varieties) and is highly supported (bs = 100%, d = 9).

Humata clade consists of all *Humata* spp. plus *D. corniculata* (bs = 99%, d = 10). The topology based on *trnL-F* is different from that based on *rbcL* in the following ways. First, Davallia clade IIa and clade IIb form a polytomy with the rest of the Davallia and Humata clades. Second, in the Humata clade, *Humata heterophylla* is a sister clade to *D. corniculata* and other *Humata* species with high support (bs = 99%, d = 10).

Bayesian analysis

The topology of the Bayesian AIC majority rule consensus tree (Figure 5.7) resulting from the analysis also shows differences from the MP tree. *Scyphularia triphylla* (included in Davallia clade IIa as sister group to *D. solida* in the MP tree) is separated from *D. solida* and is a sister clade to Davallia clade Ia and clade Ib with moderate posterior probability value (pp =0.76). The Humata clade is the same as in the MP tree. The posterior probability for the *trnL-F* region ranges between 0.59 - 1.00.

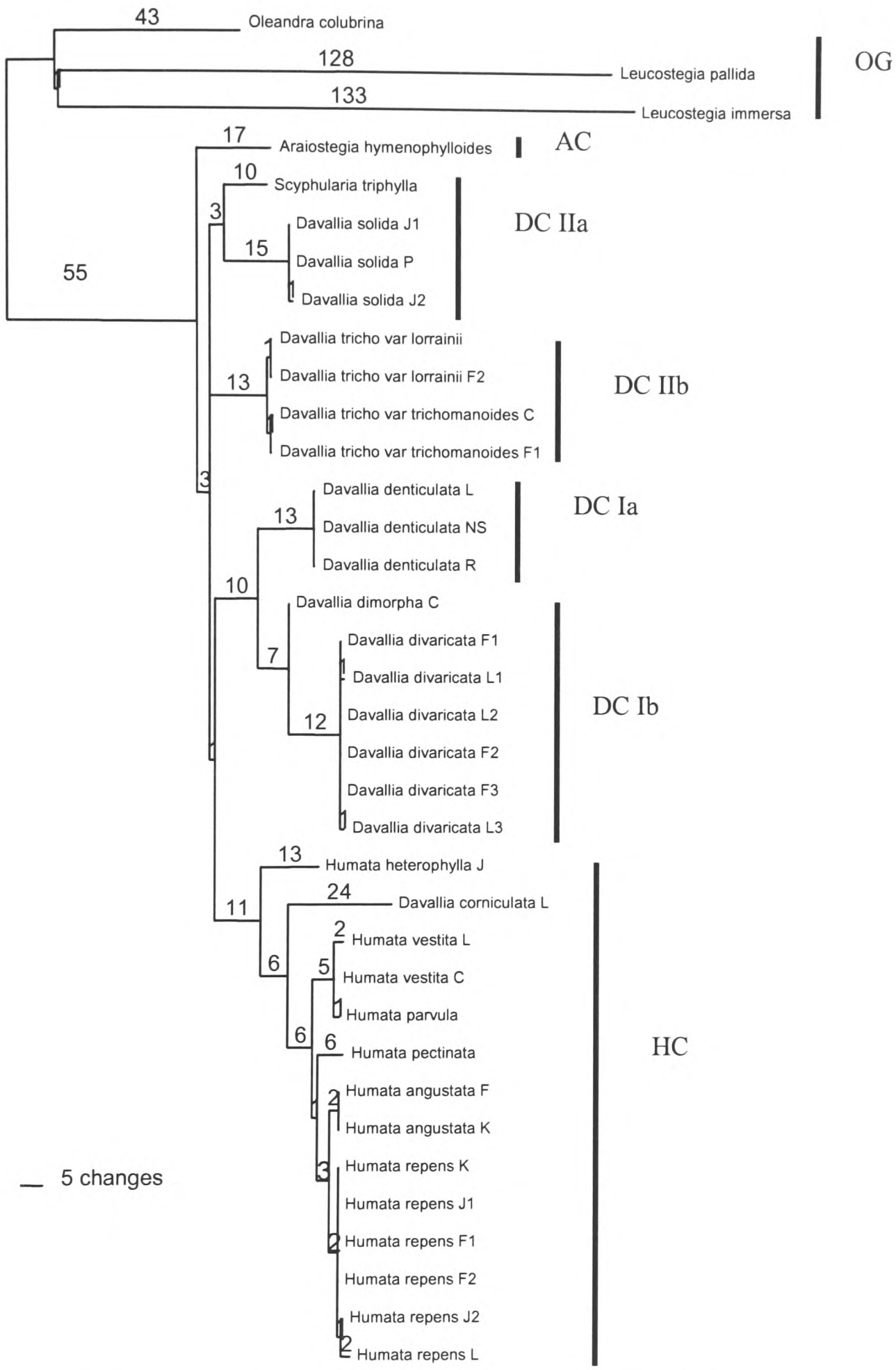


Figure 5.5: Phylogram of one most parsimony tree based on *trnL-F* sequences with a heuristic search using weighted maximum parsimony analysis of *trnL-F* including gap matrix data. (Tree length = 447 steps, CI = 0.88, RI = 0.94, RC = 0.82). The numbers above branches indicate branch length.

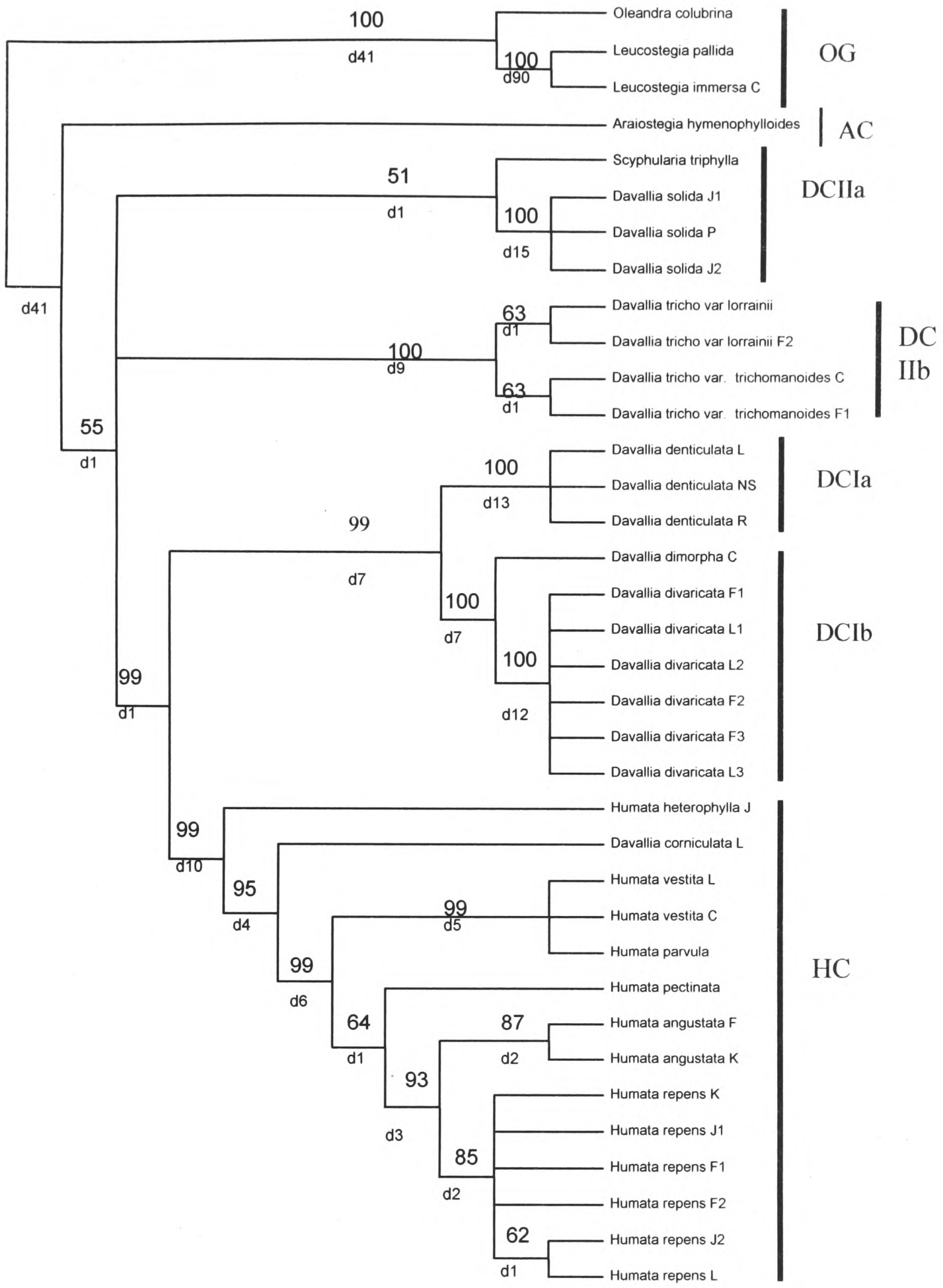


Figure 5.6: Strict consensus tree based on *trnL-F* sequences with heuristic search using maximum parsimony analysis. (Tree length = 447 steps, CI = 0.88, RI = 0.94, RC = 0.82). Numbers above branches indicate bootstrap support and numbers below branches indicate decay indices.

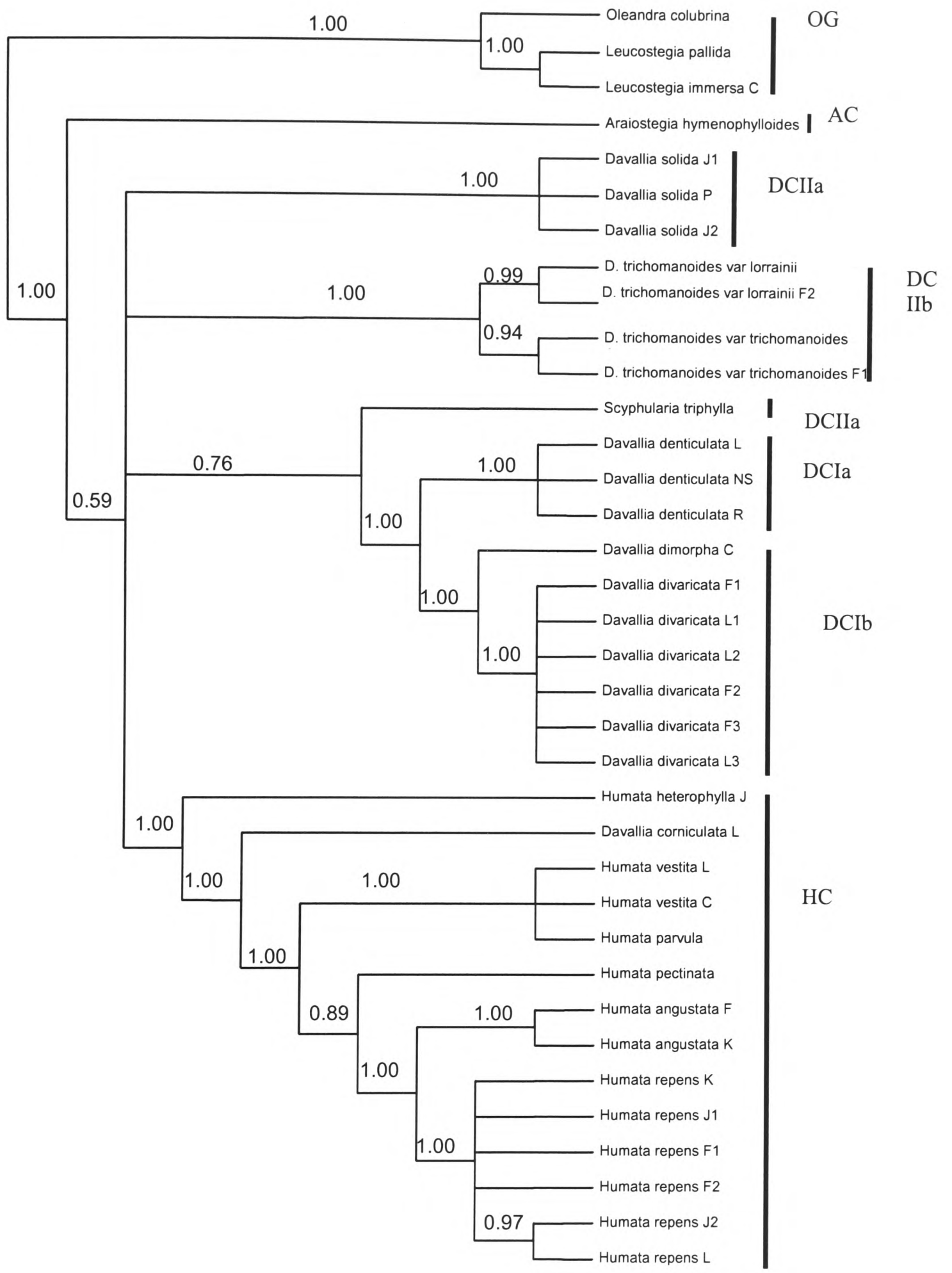


Figure 5.7: Bayesian majority rule consensus tree obtained from a *trnL-F* data matrix including gap characters. Numbers above branches are estimated posterior probability values.

5.3.4. *rps4-trnS* region

The aligment of *rps4-trnS* sequence data contains 194 informative characters out of 980 characters used. Table 5.3 summarises all the analysis information. In the maximum parsimony (MP) analysis, the first analysis using data without ambiguity produced a single most parsimony tree of 382 steps with CI = 0.84, RI = 0.93 and RC = 0.78. The second analysis using data without ambiguity and without gap characters produced a single most parsimonious tree of 374 steps with CI = 0.83, RI = 0.92 and RC = 0.77. The AIC selected model for *rps4-trnS* was GTR+G [General time reversible; Tavaré, (1986)].

5.3.4.1 **Tree topology**

Maximum Parsimony

The strict consensus tree by MP analysis (Figure 5.8 and 5.9) shows that the ingroup consists of six clades, similar to the *rbcL* and *trnL-F* topologies. The Araiostegia clade is highly supported (bs = 100%, d = 26) and forms a sister clade to all other clades. The range of bootstrap and decay values is 58% - 100% and 1 - 83 respectively. The topology based on the *rps4-trnS* region is more congruent with the *rbcL* topology compared with the *trnL-F* region. Davallia clade Ia, consisting of *D. denticulata,* and is highly supported (bs = 100% and d = 15). Davallia clade Ib also has 100% bootstrap value and d = 9. Davallia clade IIa has a bootstrap value = 75% and d = 3. Davallia clade IIb, which consists of *D. trichomanoides* (both varieties), is also fully supported (bs = 100%, d = 10). The Humata clade (including *D. corniculata*), is the sister clade to DCIIa and DCIIb, and is highly supported (bs = 96%, d = 7).

Bayesian analysis

The topology of the Bayesian AIC majority rule consensus tree (Figure 5.10) resulting from the analysis shows the same topology as the MP tree. The posterior probability value ranges between 0.52 – 1.00.

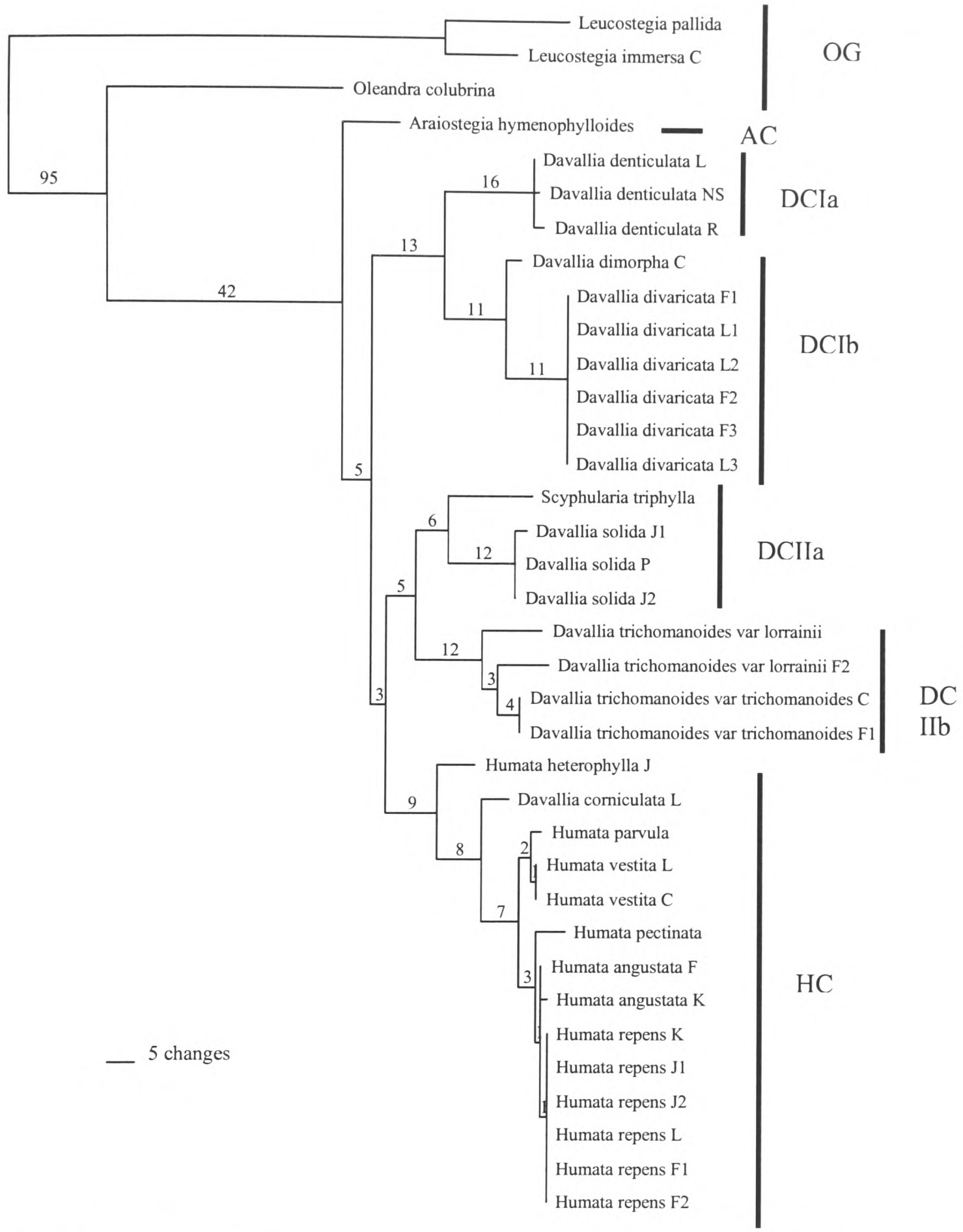


Figure 5.8: Phylogram of one most parsimony tree based on *rps4-trnS* sequences with a heuristic search using weighted maximum parsimony analysis of *rps4-trnS* including gap matrix data. (Tree length = 382 steps, CI = 0.84, RI = 0.93, RC = 0.78). The numbers above branches indicate branch length.

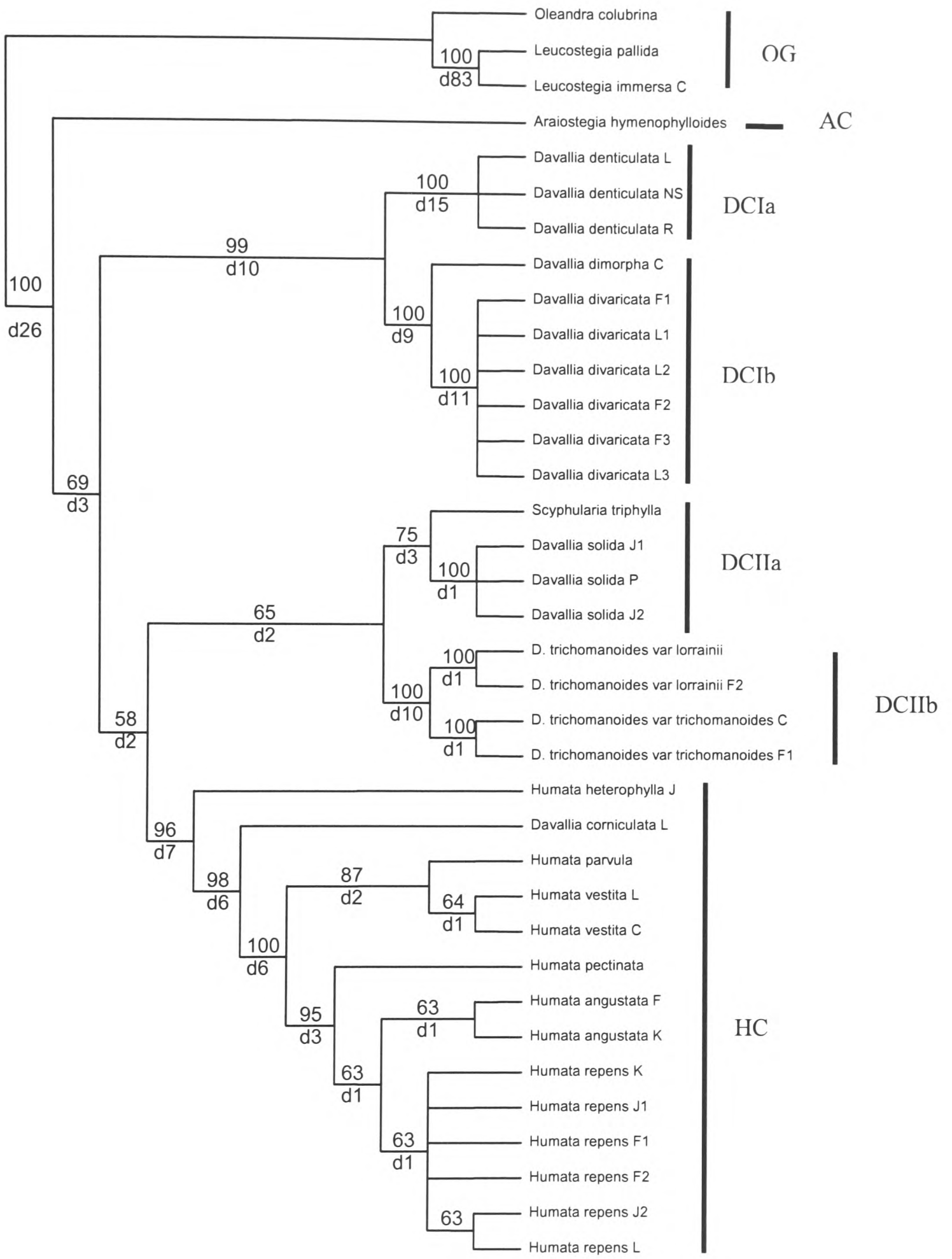


Figure 5.9: Strict consensus tree based on *rps4-trnS* sequences with heuristic search using maximum parsimony analysis. (Tree length = 382 steps, CI = 0.84, RI = 0.94, RC = 0.78). Numbers above branches indicate bootstrap support and numbers below branches indicate decay indices (d).

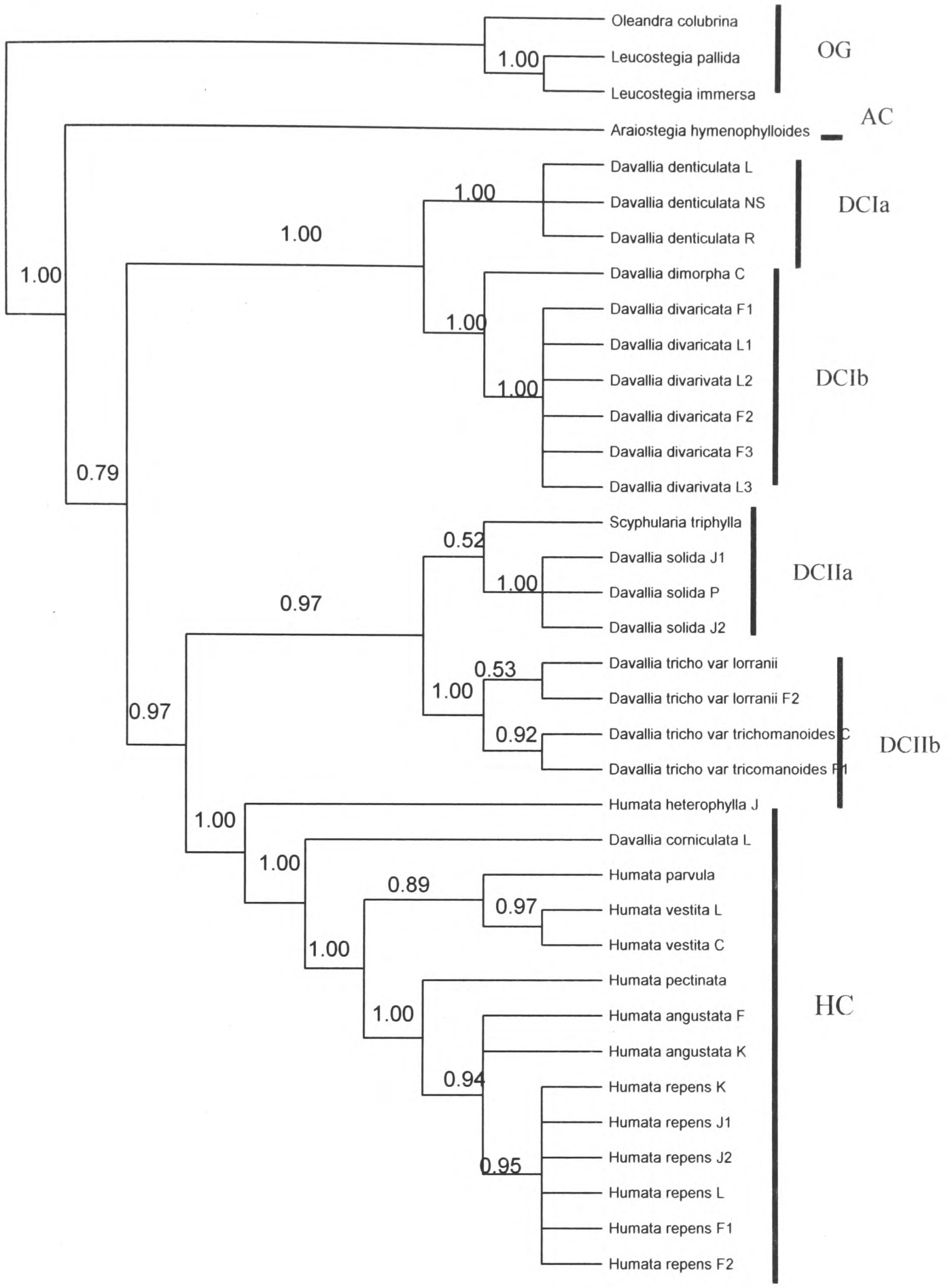


Figure 5.10: Bayesian majority rule consensus tree obtained from *rps4-trnS* data matrix including gap characters. Numbers above branches are estimated posterior probability values.

5.3.5. **Combined analysis (*rbcL* + *trnL-F* + *rps4-trnS*)**

The partition homogeneity test shows no significant incongruence between all three molecular data sets (table 5.2), and so all three data sets were combined for the analysis. The combined alignment data length for 36 taxa (33 ingroup and 3 outgroup) is 3470 characters with 23 gap characters included. The data sets reveal 219 variable but parsimony-uninformative sites and 559 that were parsimoniously informative. The *rbcL* gene contributed the least relative amount of information, with 169 variable characters of which 127 were parsimoniously informative, compared with *trnL-F* which had 331 variable characters of which 238 were parsimony informative and *rps4-trnS* with 278 variable characters of which 194 were parsimony informative.

5.3.5.1 **Tree topology**

Maximum parsimony

The analysis using combined data without ambiguity produced one most parsimonious tree (Figure 5.11 and 5.12) of 1077 steps with CI = 0.83, RI = 0.92 and RC = 0.76. The strict consensus tree of the combined analysis is fully resolved and has similar topology with *rbcL* and *rps4-trnS* which indicated six clades. Most of the relationships within the tree are highly supported. For example Davallia clade I (DCIa and DCIb), sister clade to Davallia clade II and Humata clade has 89% bootstrap value and d = 6 compare with value from *rps4-trnS region* (bs = 69 and d = 3). However the values are slightly different when compare with *rbcL* data (bs = 93% and d = 1). The bootstrap value for combined data analysis ranges between 63% to 100% (Figure 5.12.)

Bayesian analysis

The topology of the Bayesian AIC majority rule consensus tree (Figure 5.13) resulting from the analysis shows the same topology as in combined the MP tree. Again, as in MP analysis, the BA analysis indicates a high posterior probability value. Most of the values are 1.00 except in sister clade relationship between DCIIa and DCIIb which is 0.99.

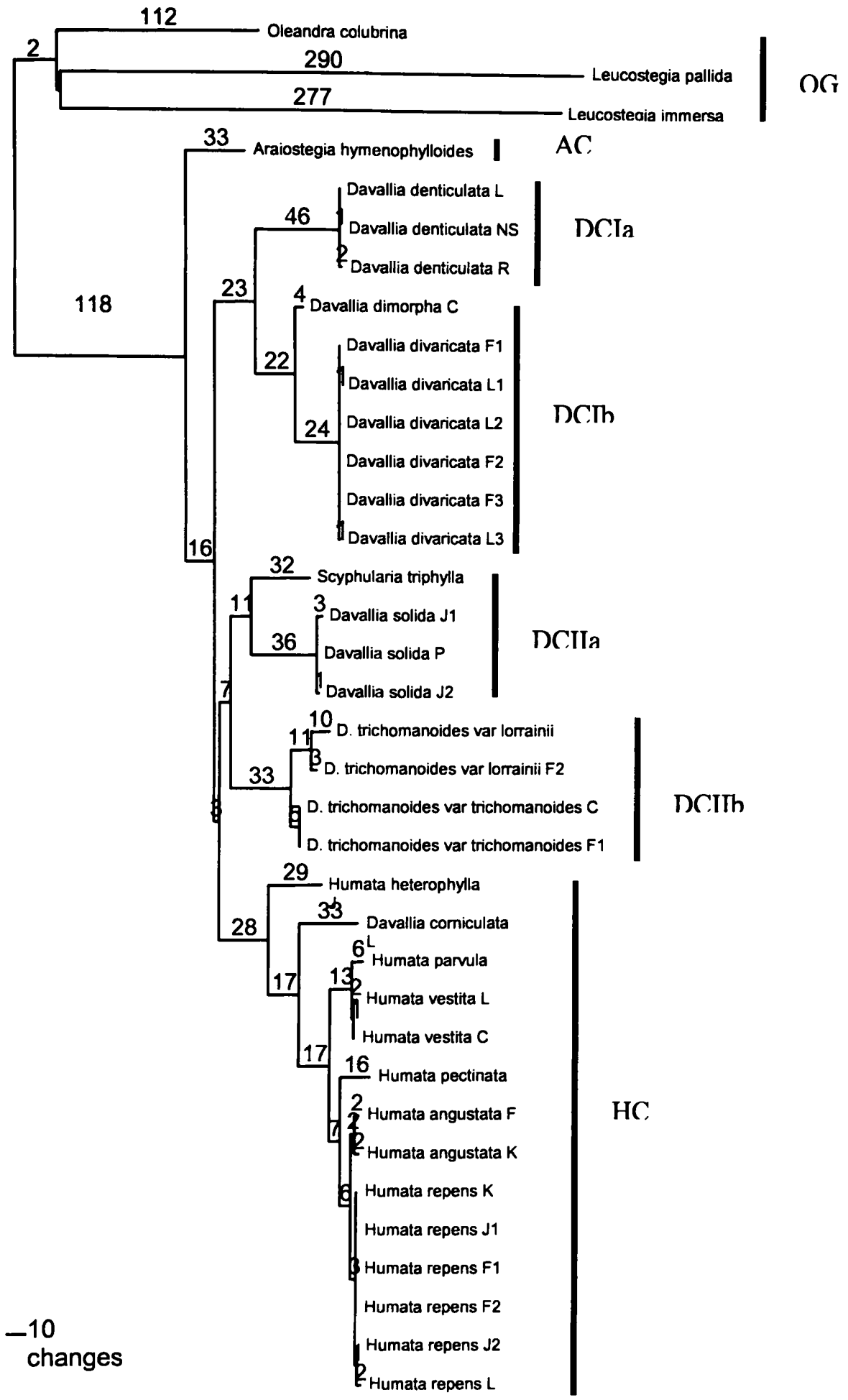


Figure 5.11: Phylogram of one most parsimony tree based on combining sequences from three regions with a heuristic search using weighted maximum parsimony analysis of combine three regions including gap matrix data. (Tree length = 1077 steps, CI=0.83, RI=0.92, RC=0.76). The numbers above branches indicate branch length.

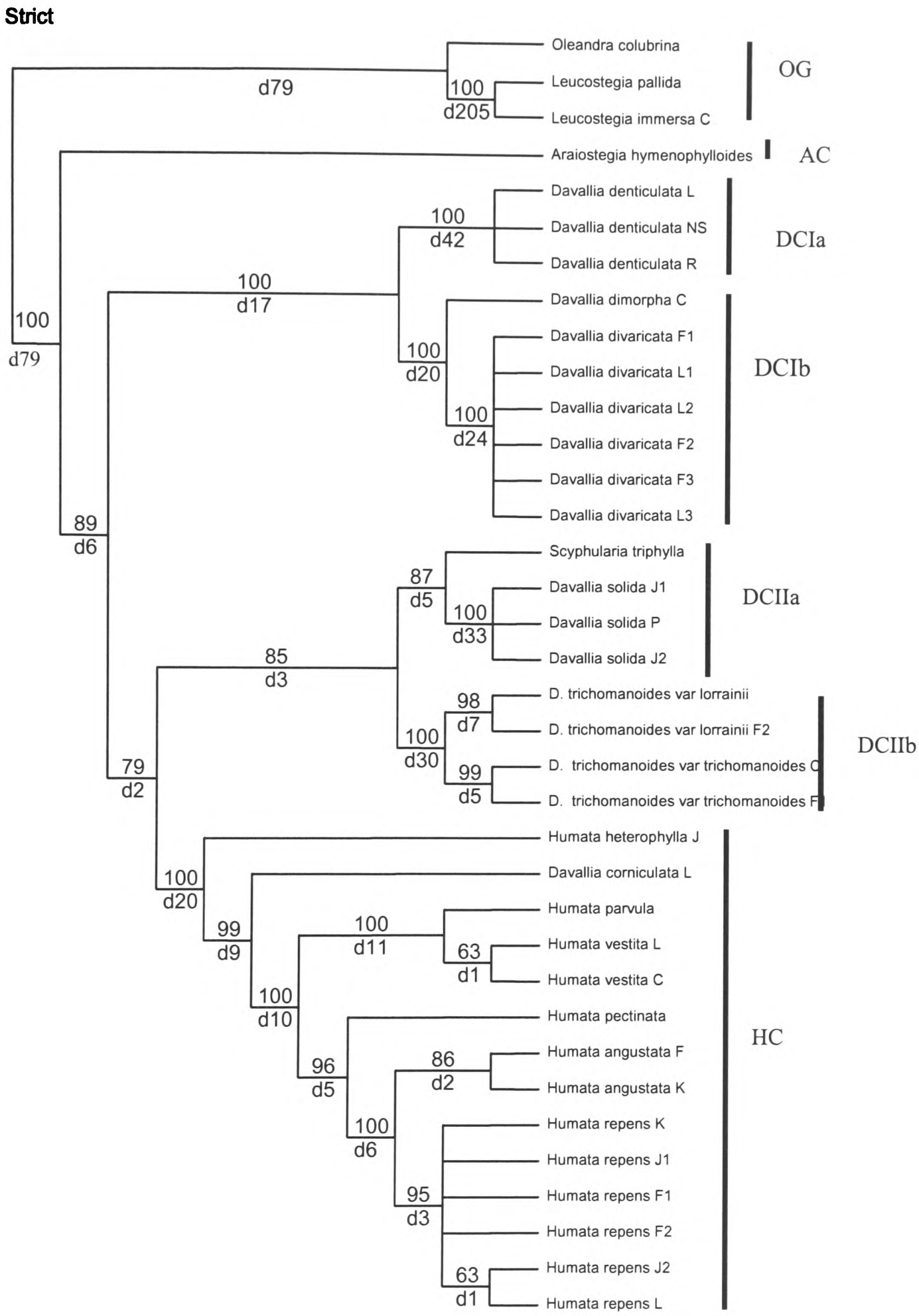


Figure 5.12: Strict consensus tree based on combining sequences from three regions with heuristic search using maximum parsimony analysis. (Tree length = 1077 steps, CI = 0.83, RI = 0.92, RC = 0.76). Numbers above branches indicate bootstrap support and numbers below branches indicate decay indices (d).

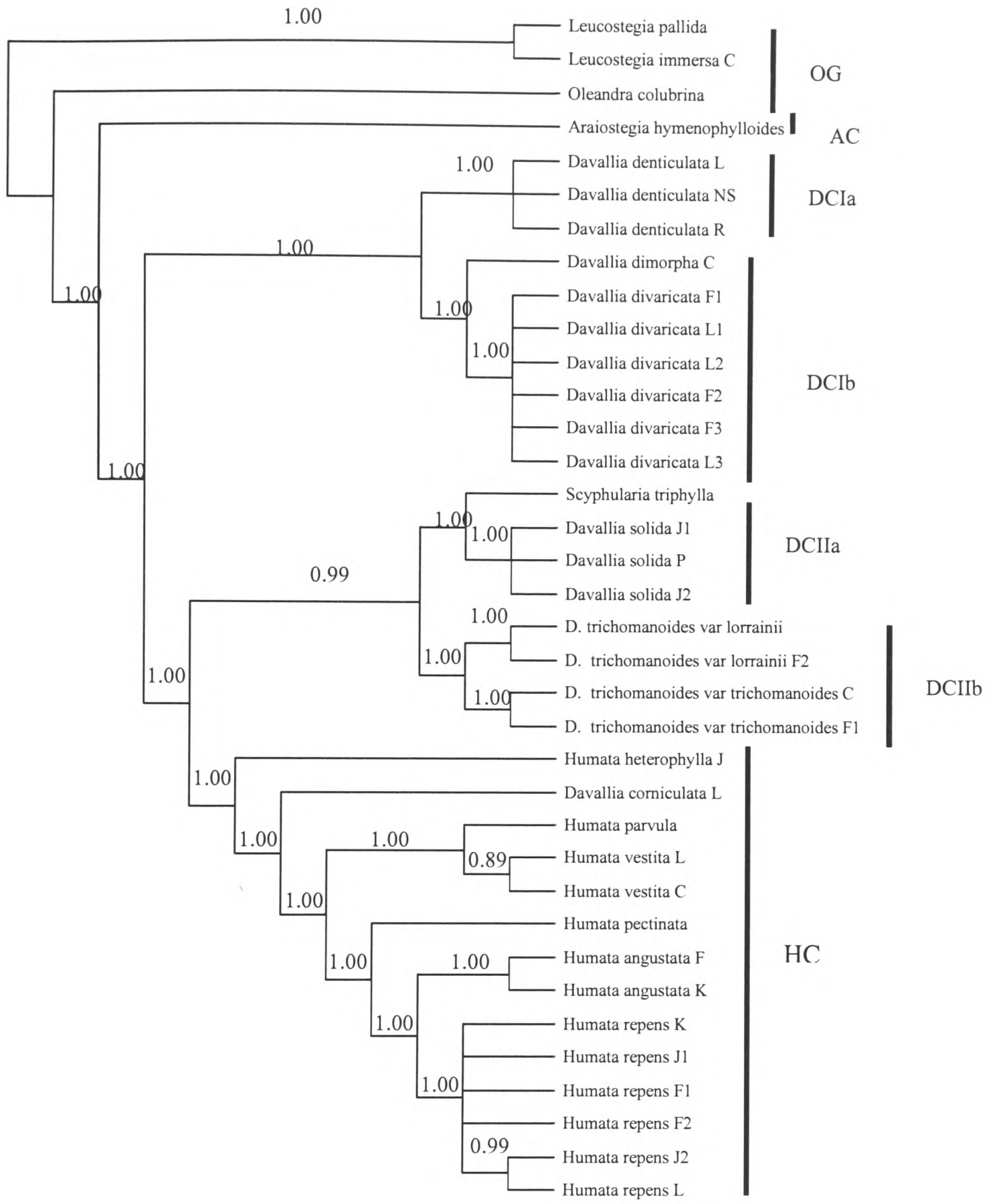


Figure 5.13: Bayesian majority rule consensus tree obtained from combining data from the three regions including gap characters. Numbers above branches are estimated posterior probability values.

5.3.6. **Expanded *rbcL* data set including sequences from Genbank**

A maximum parsimony analysis of *rbcL* data from all 36 taxa from present study including 42 *rbcL* sequences for members of Davalliaceae and two from additional outgroup species obtained from Genbank (see appendix IV) indicated that the tree was slightly different from Figure 5.2 - Figure 5.4 (tree based on 36 taxa). The consensus tree based on expanded *rbcL* indicated that the *D. denticulata* clade (DCIa) together with *Davallia embolostegia* was sister clade (weak support) to the Humata clade including *D. corniculata* (bs = < 50% and d = 0). No partition clade based on geographical region was produced (Figure 5. 14). There was consistency in the placement of multiple accessions of taxon, except *Davallia trichomanoides* (both varieties) and *Humata vestita.*

Davallia trichomanoides var. *trichomanoides* together with *D. trichomanoides* from Malaysia (Borneo) was sister clade to *D. petelotii* (Laos) and *D. trichomanoides* var. *lorrainii* with high support (bs = 90%, d = 2). Meanwhile *D. petelotii* was sister clade to *D. trichomanoides* var. *lorrainii* (bs = 100%, d = 6; Figure 5.15). *Araiostegia hymenophylloides* with *A. pulchra*, *A. yunnanensis* and *Davallodes* spp formed a basal clade and a sister clade to all other ingroup taxa including *A. perdurans* and *A. faberiana.*

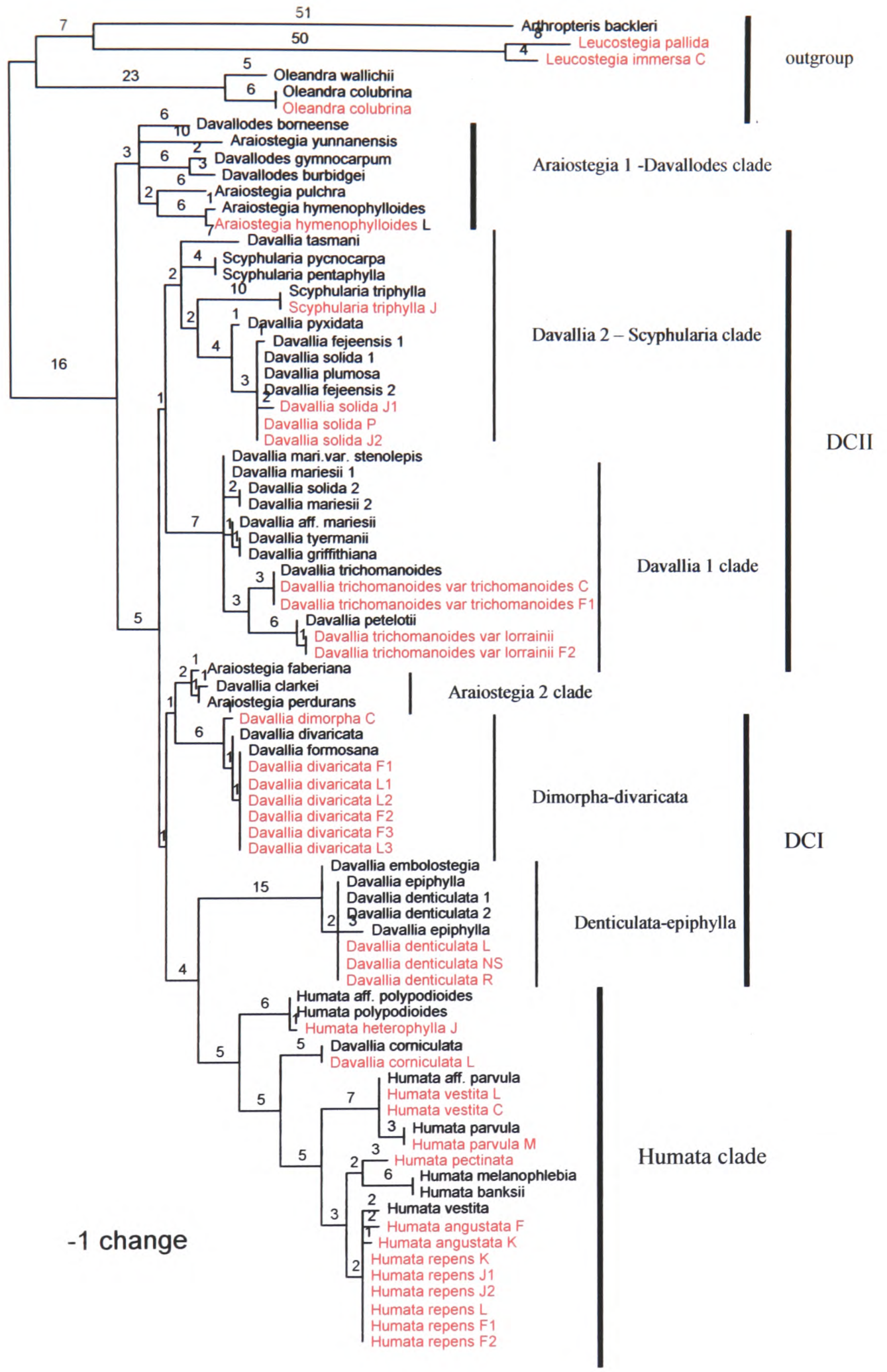


Figure 5.14: Phylogram of one of five most parsimony tree base on *rbcL* (81 taxa) sequences with a heuristic search using weighted maximum parsimony analysis of the combined three regions including gap matrix data. (Tree length = 361 steps, CI=0.65, RI=0.89, RC=0.58). The numbers above branches indicates branch length. The red font taxa indicate samples from this study.

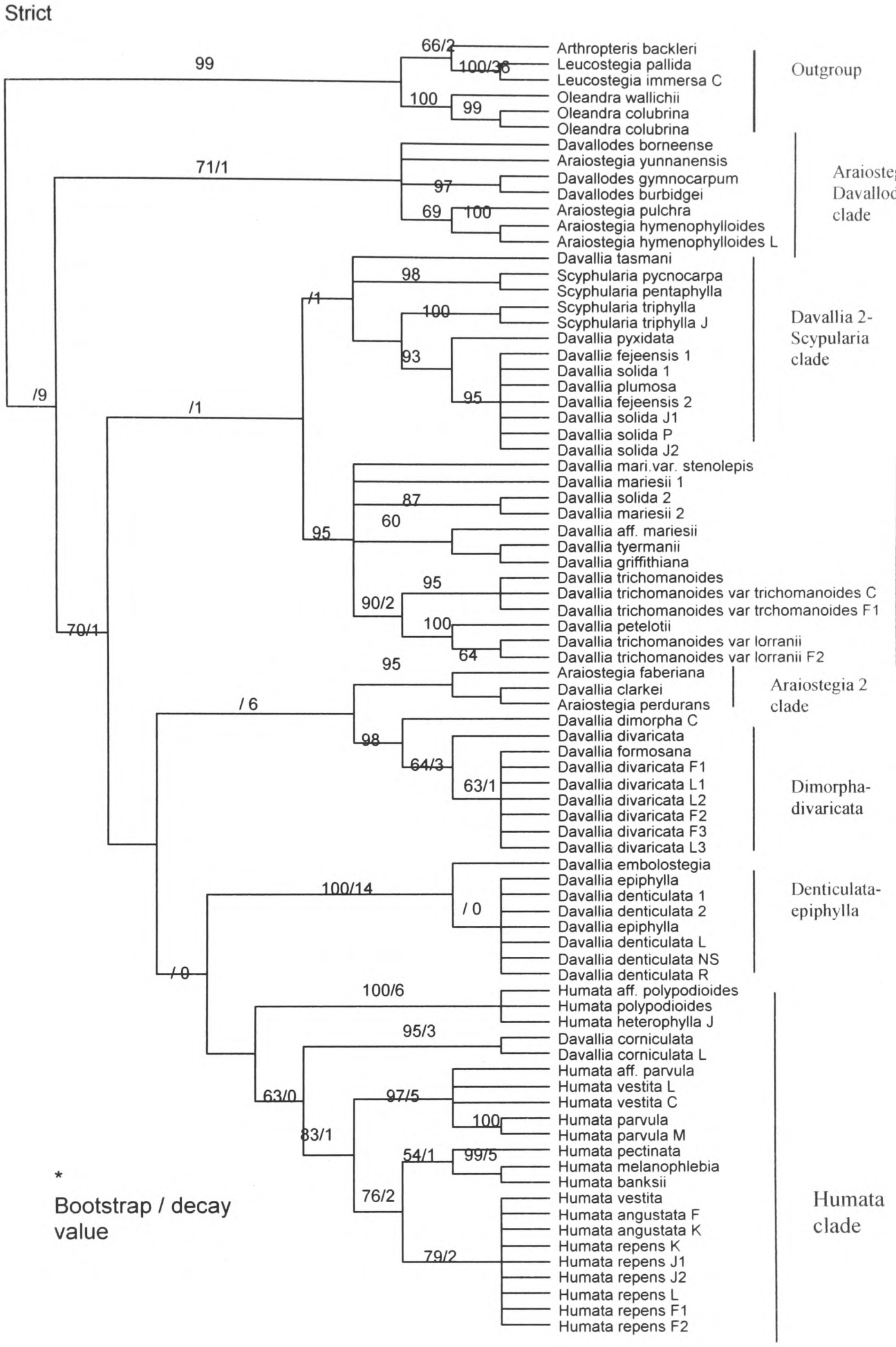


Figure 5.15. Strict consensus tree based on expanded *rbcL* data with heuristic search using maximum parsimony analysis. (Tree length = 361steps, CI = 0.65, RI = 0.89, RC = 0.58). Numbers indicated bootstrap and decay value.

5.4 **Discussion**

5.4.1 **The Monophyly of Davalliaceae.**

The phylogenetic relationships based on three chloroplast regions data sets were incongruent with any previous morphological classification and indicates that neither of the large genera within Davalliaceae, i.e. *Humata* and *Davallia* are monophyletic. In this respect it supports the findings of Tsutsumi and Kato (2005). In phylogenetic studies using molecular data as characters, the delimitation between the different states is easy to determine as these characters are different, discrete and no intermediate states exist (Pennington, 2000). The suitability of the *rbcl, trnL-F* and *rps4-trnS* regions has already been tested in other studies on fern phylogeny (Schneider et al., 2004a; 2004b; Skog et al., 2004). In this study these three regions show a clear resolution of phylogenetic relationships within Davalliaceae species. The trees show that Davalliaceae (excluding *Leucostegia*) comprises six clades, namely the Araiostegia clade (AC), four Davallia clades (DCIa, DCIb, DCIIa and DCIIb) and the Humata clade. The partitioning of clades are almost the same in all trees based on these three regions although there is a slight difference seen in the *trnL-F* tree. The significance of all clades was estimated using boostrap and posterior probability value.

According to Richardson et al. (2000), a bootstrap value between 50% - 74% is considered as weak support, 75% - 84% as a moderate support and above 85% is strongly supportive. A posterior probability value (pp) more than 95% is considered as significant (Wilcox et al., 2002; Alfaro et al., 2003). Alfaro et

al. (2003) also proposed that these statistics should be seen as measuring different features of the data and results with very high posterior probabilities but moderate bootstrap value should be interpreted as having a high probability of being correct. It is therefore not possible to decide which estimate of phylogenetic confidence is most accurate without knowing the true phylogeny.

5.4.2 *rbcL* region analysis

The topology from the *rbcL* data analysis is highly resolved (bs > 80%). *Araiostegia* forms its own clade and is a sister clade to all Davallia clades and the Humata clade with high support (bs = 100%, pp = 1.00). *Davallia* and *Humata* form four clades and a single clade respectively (bs >80%). Davallia clade Ia consists of *D. denticulata* and is a sister clade to Davallia clade Ib (bs = 100%). Davallia clade Ib consists of *D. dimorpha*, a sister clade to *D. divaricata*, also with a high bootstrap value. Both these species share the same morphological characters of tri-quadripinnate fronds, dimorphic fronds and large rhizome. However the BA analysis does not support the relationship between the Davallia clade Ia and Davallia clade Ib. It places the Davallia clade Ia in a polytomy with the Araiostegia clade (pp = 1.00). Davallia clade IIb consists of *D. trichomanoides* with high bootstrap and posterior probability value (bs = 100%, pp = 1.00). However the relationship between Davallia clade IIa and Davallia clade IIb is moderately supported (bs = 85%, pp = 0.86). The Humata clade consists of *D. corniculata* as a sister clade to all other *Humata* species (bs = 100%, pp = 1.00). The presence of *D. corniculata* within

the Humata clade maybe result from the shared morphological characters of small white waxy rhizome, coriaceous leaves and shell shaped indusia, which are found in many *Humata* spp.

In general, the resultant tree on *rbcL* analysis is similar to the study done by Tsutsumi and Kato (2005). *Araiostegia* remains as the basal lineage within Davalliaceae. Davallia clade Ia and Ib refer to DC 3 in Tsutsumi and Kato's study. Clade IIa in the current study refers to DC 2 and the scyphularia clade and DC IIb refers to DC 1 in Tsutsumi and Kato (2005).

5.4.3 ***trnL-F* region analysis**

Topology based on the *trnL-F* sequence data also results in six clades. However the sister relationship of *Araiostegia*, to all other clades, is weakly supported with bs value <50%. In both analyses, DCIIa and DCIIb form a polytomy with other clades. DCIa is a sister clade to DCIb, which is highly supported (bs = 99%, d=7). The Humata clade shows a slight change topologically compared with the *rbcL* tree. *Humata heterophylla* is a sister clade to all other *Humata* species, including *D. corniculata,* with high support (bs = 99%, d=10). The relationship between all Davallia clades and the Humata clade is weakly supported in Bayesian analysis (pp = 0.59). This analysis also shows that *Scyphularia* and *D. solida* (DCIIa) are not sister taxa, with *Scyphularia* being a sister clade to DCIa and DCIb (Figure 5.7). Meanwhile *D. solida* forms a polytomy with other clades. As no study has

been done on the same gene in other members of Davalliaceae, comparison with other data could not be made.

5.4.4 *rps4-trnS* region analysis

Topology based on MP and BA is fully resolved as with *rbcL*. Six clades still persist. *Araiostegia* forms a sister clade to other clades and is highly supported (bs = 100%, d = 26 and pp = 1.00). The relationship of DCIa and DCIb is strongly supported (bs = 99%, pp = 1.00). However, the monophyly of each of DCIIa, DCIIb and the Humata clade have weak bootstrap support (bs = 58 – 65%) but strong posterior support (pp = 0.97). The Humata clade remains the same as shown in the *trnL-F* tree, whereas *H. heterophylla* is a sister clade to all other *Humata* species (including *D. corniculata*). This result could not be compared with other data as no previous study has been done on the same gene.

5.4.5 Combined (*rbcL, trnL- F and rps4-trnS*) analysis

Topology based on the combined analysis is fully resolved. *Araiostegia* remains as the basal clade sister to all other clades (bs = 100%, pp = 1.00). The monophyly of a clade comprising DCIa, DCIb, DCIIa, DCIIb and the Humata clade in MP is moderately supported (bs = 89%). However in BA it is highly supported (pp = 1.00). In the Humata clade, *H. heterophylla* remains as sister clade to all other *Humata* species plus *D. corniculata*, as in the analyses of *trnL-F* and *rps4-trnS* regions.

5.4.6 **Expanded *rbcL* data**

Analysis based on *rbcL* for all taxa (present study and Genbank) showed that all the species collected in Peninsula Malaysia were fully resolved within the clades recognized by Tsutsumi and Kato (2005; Figure 5.1), not as different regional clades. There was no conflict shown in the positioning of taxa except the placement of DCIa including *D. embolostegia* plus the *D. epiphylla* clade as a sister clade to the Humata clade. *Araiostegia hymenophylloides* and the *Davallodes* clade persisted as the basal clade to all other species of Davalliaceae.

5.4.7 **Phylogeny based on molecular and morphology**

Systematists have traditionally treated morphological and molecular data as distinct data sets but such division may not always be realistic (Kluge and Wolf, 1993). The present study showed that analysis of combined sequence and morphological data was fully resolved as seen in the strict consensus tree (Figure 5.16). The tree also indicated the genera of Davalliaceae were paraphyletic. It consists of *Araiostegia* as a basal clade, Davallia Clade Ia (*D. denticulata*), Davallia clade Ib (*D. dimorpha* and *D. divaricata*), Davallia Clade IIa (*Scyphularia triphylla* and *D. solida*), Davallia clade IIb (*D. trichomanoides* var. *lorrainii* and *D. trichomanoides* var. *trichomanoides*) and Humata clade (all *Humata* species + *D. corniculata*).

Most of clades and sister relationships in Davalliaceae were strongly supported (bs = >85%) excepts 70 % support for DCII and the Humata clade being sister clade and 69% support for a clade comprising DCIIa and DCIIb.

5.5 **Conclusions**

Although the tree based on *trnL-F* was slightly different, topology based solely on the coding region *rbcL*, non coding region *rps4-trnS* and combined data sets proved that none of the genera of Davalliaceae was monophyletic, as suggested by Tsutsumi and Kato. Hence with additional molecular information, the previous classification which lumped all *Davallia* and *Humata* species in one genus should be reviewed; a suggested alternative would be either to accept each clades as a one genus, with *D. corniculata* incorporated in *Humata* or partially agree with Nooteboom by grouping *Davallia* and *Humata* in one genus except *Araiostegia* and subdivide the genus into three different sections instead of two; Davallia, Scyphularia and Humata (plus *D. corniculata*) based on clade formation in this study. In addition, *Leucostegia* should be removed from Davalliaceae.

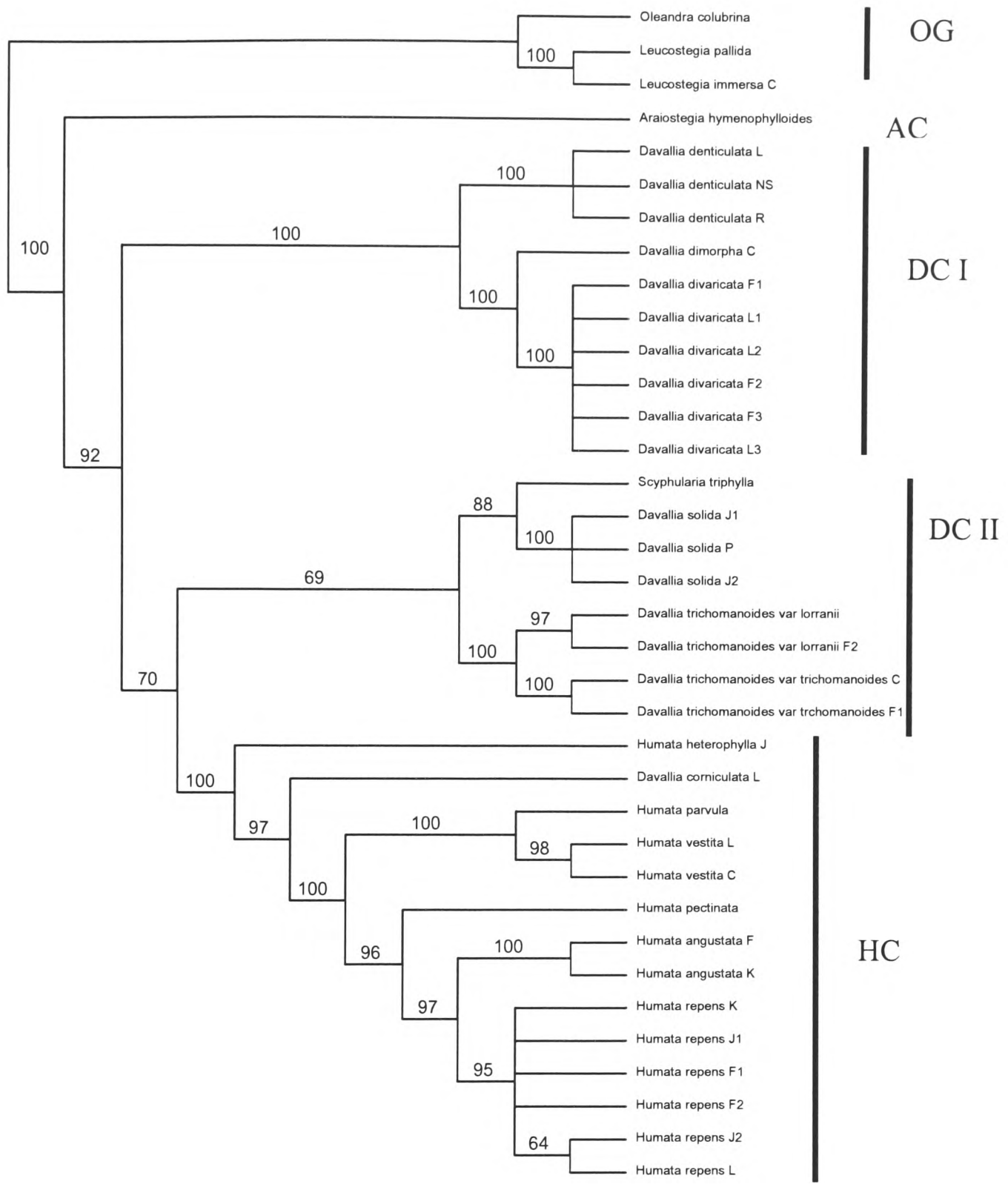


Figure 5.16. Strict consensus tree based on combined sequence and morphological data with heuristic search using maximum parsimony analysis. (Tree length = 1141; CI = 0.81; RI = 0.91; RC = 0.73). Numbers above branches indicate bootstrap support.

Chapter Six

Morphological character mapping and the evolution of epiphytism

6.1 Introduction

The availability of a well-resolved and well-supported phylogeny for Davalliaceae based on both morphological and molecular provides an opportunity to examine the evolution of morphological characters within the family. Of particular interest were the traits that might be of ecological relevance; in particular those that relate to life forms. Davalliaceae comprises epiphytes, lithophytes and terrestrial plants, and some species exhibit more than one of these habits.

The name epiphyte is derived from Greek, and was introduced by Mirbil in 1815 (Nieder et al., 1999). The prefix `epi' means `upon'- an epiphyte is a plant that grows upon another plant. It is not a parasite and does not directly obtain nutrition from the host tree. Epiphytes are characteristic of tropical forest and oceanic islands, where there is year-round high energy level and high humidity. There are about 900 genera and almost 30,000 species of epiphytes in the world but no family is totally epiphytic. In monilophytes (ferns, sensu Pryer et al., 2004) about 25% of extant ferns (9,000 species) are epiphytic. Polypodiaceae is the largest fern family (ca. 1200 spp) and contains the most epiphytic species followed by Aspleniaceae (ca. 700 spp) and Hymenophyllaceae (ca. 600 spp).

Davalliaceae is a smaller family (ca. 50-130 spp), mostly consisting of epiphytic species.

In a broad sense, epiphytes are classified into three types, as true or obligate epiphytes, facultative epiphytes and hemi-epiphytes (Benzing, 1989). They may also be classified in several different ways, for example by size, morphology, ecology, physiology or by combination of these methods (Ingrouille & Eddie, 2006). In ferns, Holttum (1938) classified epiphytic ferns as lower and high epiphytes. Recently, the terms `hemi-epiphytes' and `true epiphytes' were used in ferns, e.g. in Hymenophyllaceae (Dubuisson et al., 2003) and in Davalliaceae (Tsutsumi and Kato, 2006).

Epiphytism has probably evolved independently from terrestrial stock in most families of ferns (Benzing, 1987). In the first review of the ecological diversity of the genus *Trichomanes* (Hymenophyllaceae), Dubuisson et al. (2003) suggested that, in *Trichomanes*, true epiphytes evolved from terrestrial ancestors or hemi epiphytes. Tsutsumi and Kato (2006) also claimed that true epiphytes were derived from hemi epiphytes based on field observations and molecular phylogenetic analyses in Davalliaceae. In this chapter, the combined phylogeny based on molecular and morphological data was used to examine the evolution of morphological characters and habits within the family, asking whether they evolved once or multiple times, defining the ancestral state for each character, and identifying diagnostic characters where they exist for the identified clades.

6.2 **Materials and methods**

Morphological characters associated with life form of all species collected were identified and noted during field work, including information on characters of the rhizome (e.g. size) and connection of species with soil (Table 3.1). Each character was mapped onto the topology based on the combined morphology and molecular data sets using Mac Clade 4.06 (Maddison and Maddison, 2003) to observe character evolution.

6.3 **Results**

6.3.1 **Field observations**

a. **Distribution and habitat**

In Peninsular Malaysia, Davallioid ferns are widely distributed. The most common species are *Davallia denticulata* and *Humata repens*. *Davallia denticulata* was distributed throughout lowland forest, palm plantations and exposed seashore. Thus this species could survive in both shaded and exposed areas. However, *H. repens* was found almost exclusively in shaded areas. This species exhibited much variation in size of frond. All the other species were distributed in shaded areas of lowland or mountainous regions except *H. parvula* which was found creeping on mangroves.

b. **Life form**

Based on specimens collected during field work, the life forms or habit of Davallioid ferns in Peninsular Malaysia comprise four types, true epiphytes, hemi epiphytes, lithophytes and terrestrial plants. Most of the species collected

were true epiphytes. These were *Leucostegia immersa, L. pallida, Davallia denticulata, D. divaricata, D. dimorpha, D. solida, D. trichomanoides* var. *lorrainii* and *Humata parvula*. *Leucostegia pallida* and *Humata parvula* were not found during fieldwork but were cited in the literature (e.g. Holttum, 1968) and herbarium specimens as being true epiphytes. *Scyphularia triphylla*, found on the basal part of a higher plant was also classified as an epiphyte. Some davallioid species were found to be creeping on mossy rock (lithophytes), for example, *Araiostegia hymenophylloides* (although this was also found as a terrestrial plant), *H. angustata* and *H. pectinata*. Terrestrial life form was observed in *H. vestita*, *H. repens* (also found on rock), *D. corniculata* and *D. trichomanoides* var. *trichomanoides*. *Oleandra* (outgroup) was identified as a hemi epiphyte as it was found creeping up from the basal part of the tree (approximately two to three feet high) and had connection with the soil. Mapping the life form character onto phylogenetic tree (Figure 6.1) showed that most of the species (true epiphytes) were derived from hemi epiphytes (ancestral state). It also suggested that the terrestrial habit evolved independently in *D. trichomanoides* var. *trichomanoides, D. corniculata, Humata vestita* and in *Humata pectinata*.

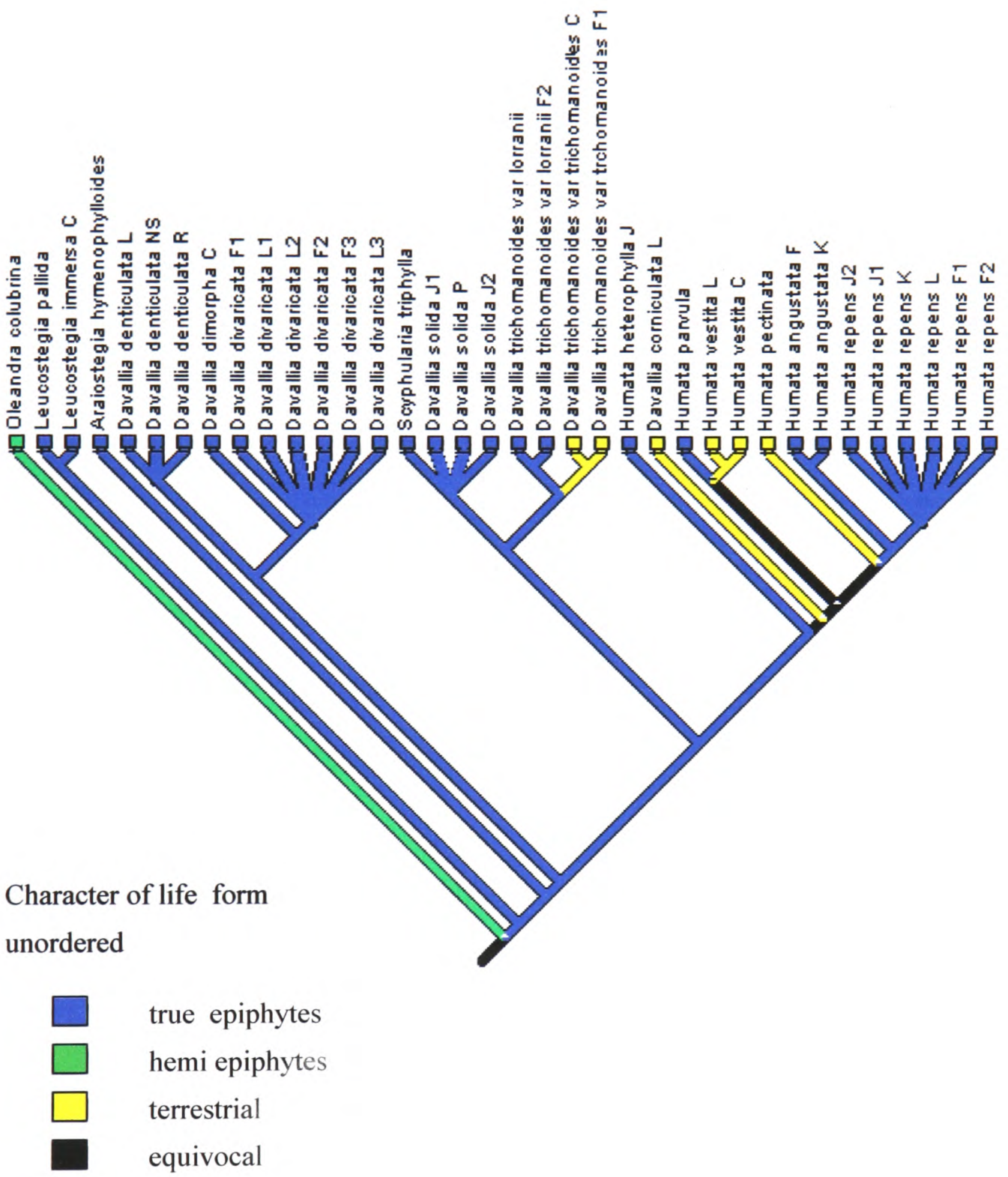


Figure 6.1: Inferred evolution of epiphytes in Davalliaceae through mapping life form characters onto the phylogenetic tree based on combined three regions.

6.3.2. Morphological character evolution based on mapping onto molecular tree

In all trees, most morphological characters evolved more than once and hence exhibited some homoplasy. They were mostly shared over a wide range of ingroup and outgroup taxa and most were not found to be a unique character for one clade. The evolution of characters 2 (waxy rhizome), 3 (rhizome shape), 6 (scale attachment), 9 (stipe surface), 13 (frond structure), 15 (sori location), 16 (indusium structure), 17 (indusium attachment) and character 19 (indusium shape) were shown in Figure 6.2 -6.6.

1. **Character no. 2 – white waxy rhizome** (Figure 6.2)

Ancestral: No waxy layer.

Number of changes: one in Humata clade.

2. **Character no. 3 - Rhizome shape** (Figure 6.2)

Ancestral: rounded or slender shape.

Number of changes: one in *Scyphularia triphylla*

3. **Character no. 6 - Scales attachment** (Figure 6.3)

Ancestral: Peltate scale

Number of changes: two in *Araiostegia hymenophylloides*, *D. dimorpha* and *D. divaricata.*

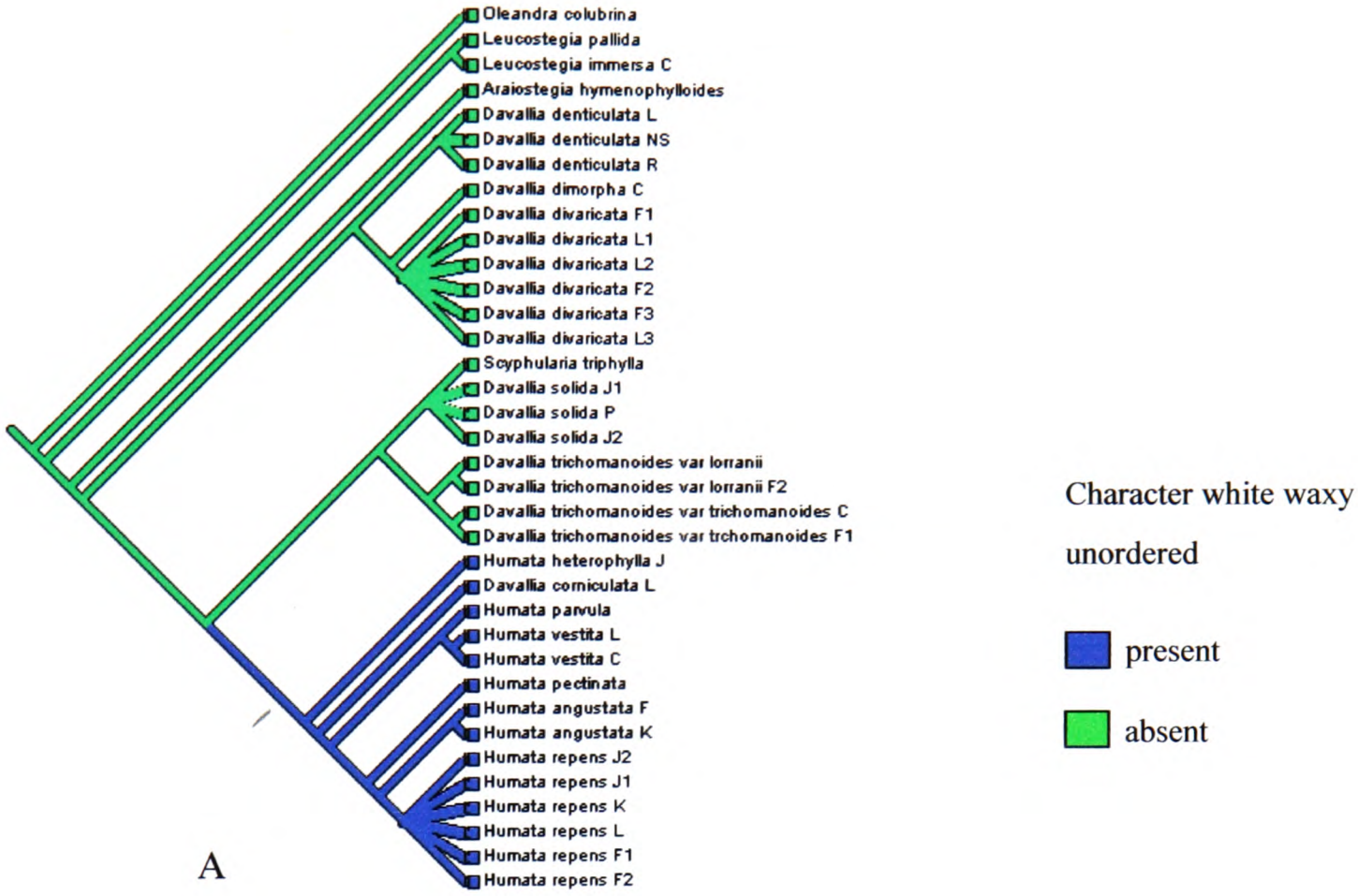


Figure 6.2: Inferred evolution of white waxy (A) and rhizome shape (B) via mapping the characters onto the phylogenetic tree

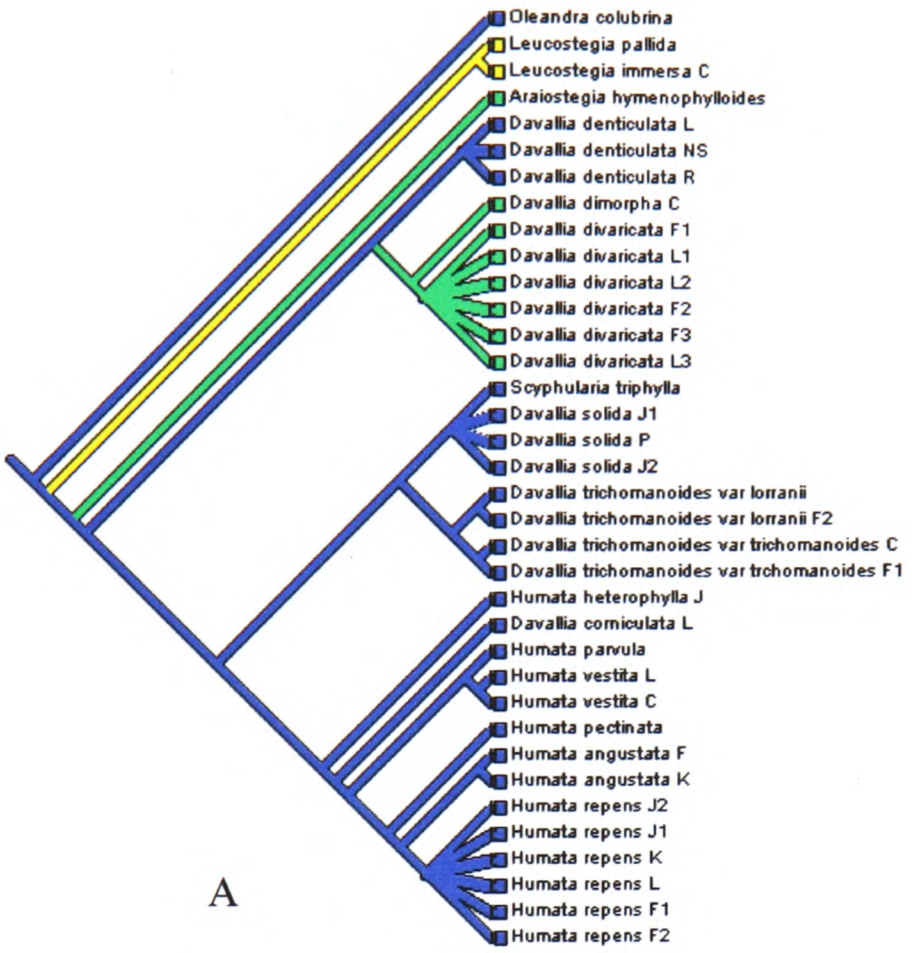


Figure 6.3: Inferred evolution of scale attachment (A) and stipe (B) via mapping the characters onto the phylogenetic tree

4. **Character no. 9 – Stipe** (Figure 6.3)

Ancestral: glabrous stipe

Number of changes: one

5. **Character no. 13 - Frond dimorphism** (Figure 6.4)

Ancestral: monomorphic frond

Number of changes: four in *D. dimorpha*, *D. divaricata*, *H. heterophylla*, *H. parvula*, *H. vestita* and *H. angustata*.

6. **Character no. 15 – Sori location** (Figure 6.4)

Ancestral: near midrib

Number of changes: three

7. **Character no. 16 - Indusium structure** (Figure 6.5)

Ancestral: glabrous indusium

Number of changes: one

8. **Character no. 17 - Indusium attachment** (Figure 6.6)

Ancestral: basal attachment

Number of changes: three.

In *D. corniculata* (Humata clade), the transition to base and partial side attachment evolved independently.

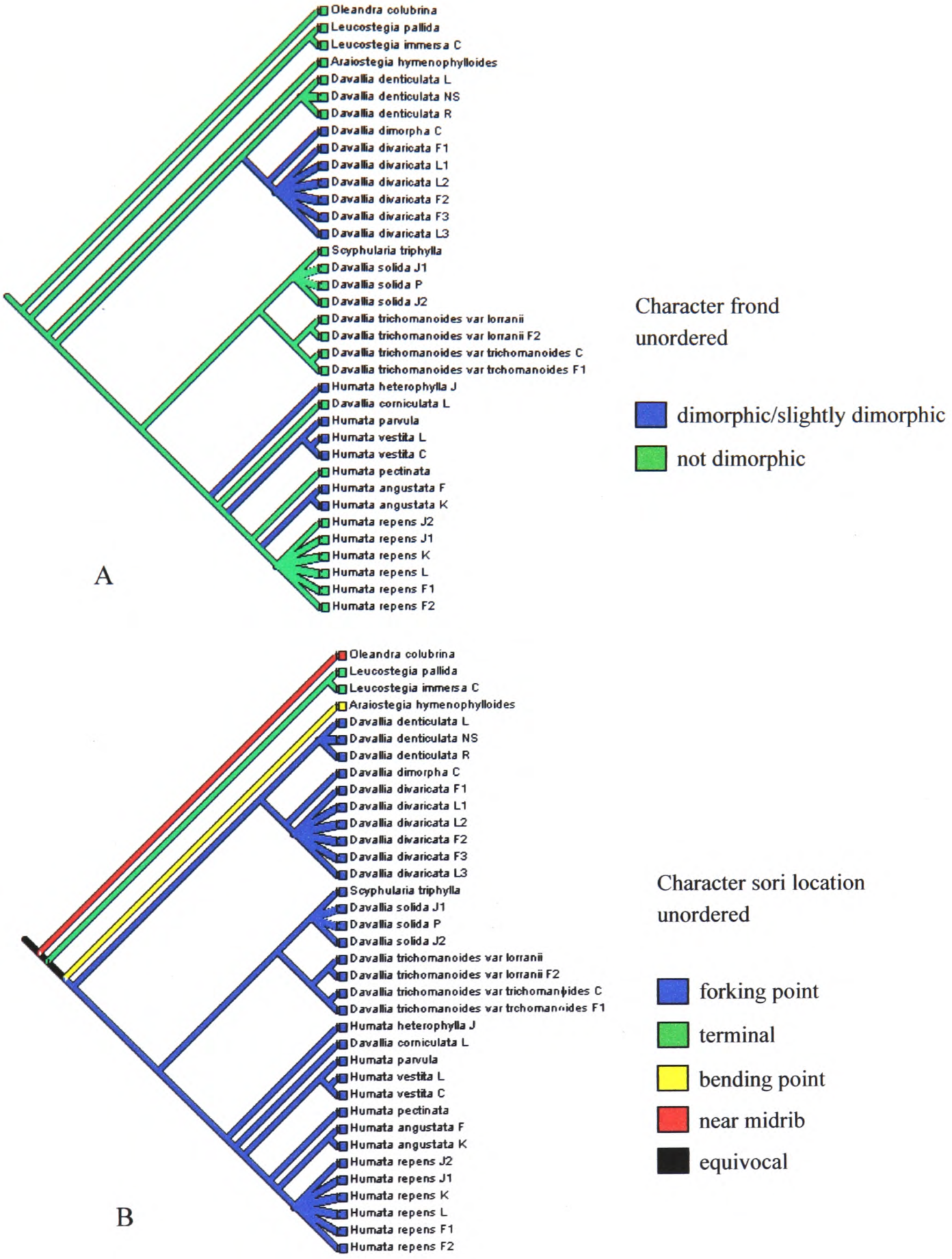


Figure 6.4: Inferred evolution of frond (A) and sori location (B) via mapping the characters onto the phylogenetic tree

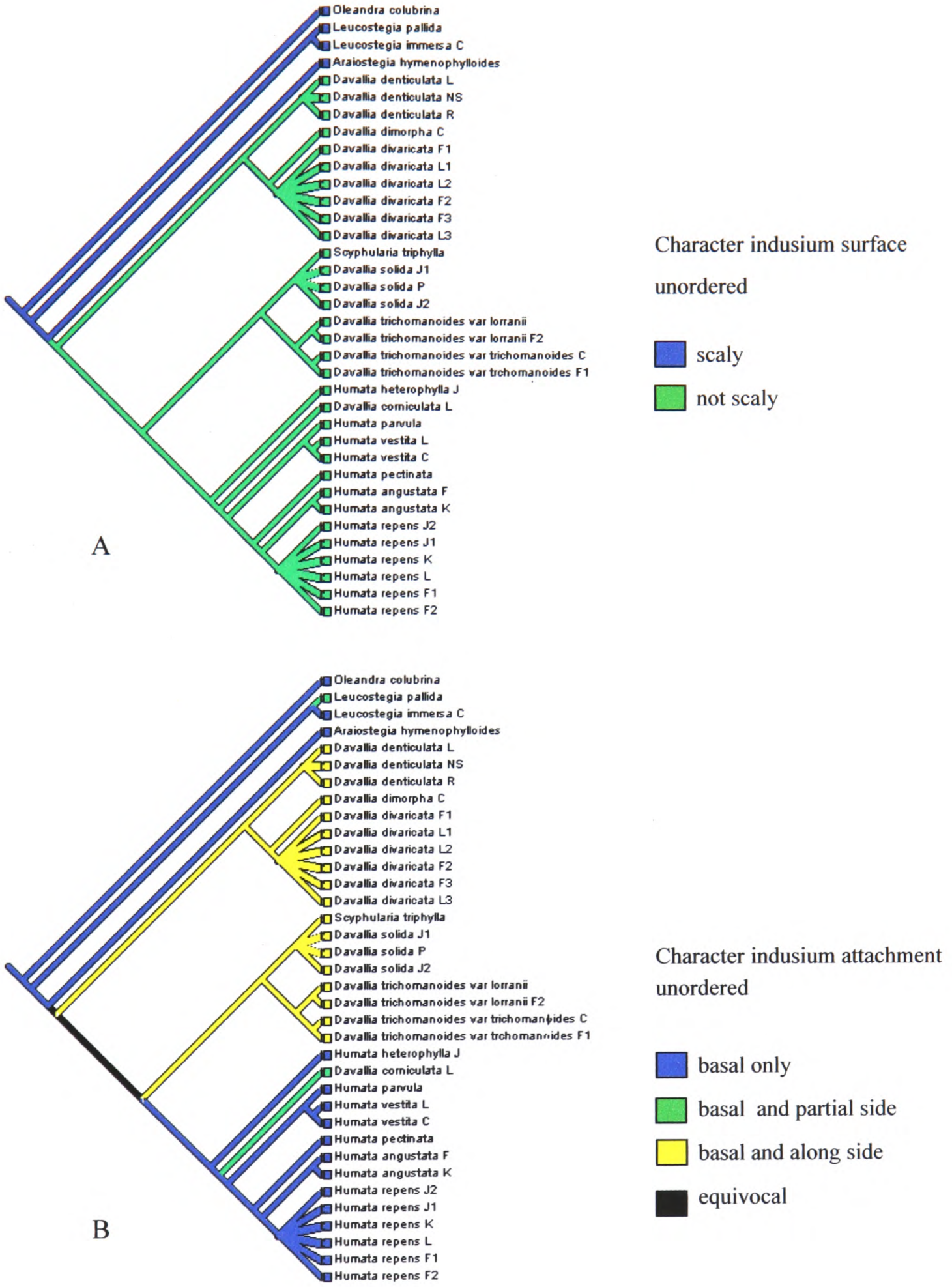


Figure 6.5: Inferred evolution of indusium structure (A) and indusium attachment (B) via mapping the characters onto the phylogenetic tree.

9. **Character no 19 – Indusium shape** (Figure 6.6)

Ancestral: reniform

Number of changes: at least three.

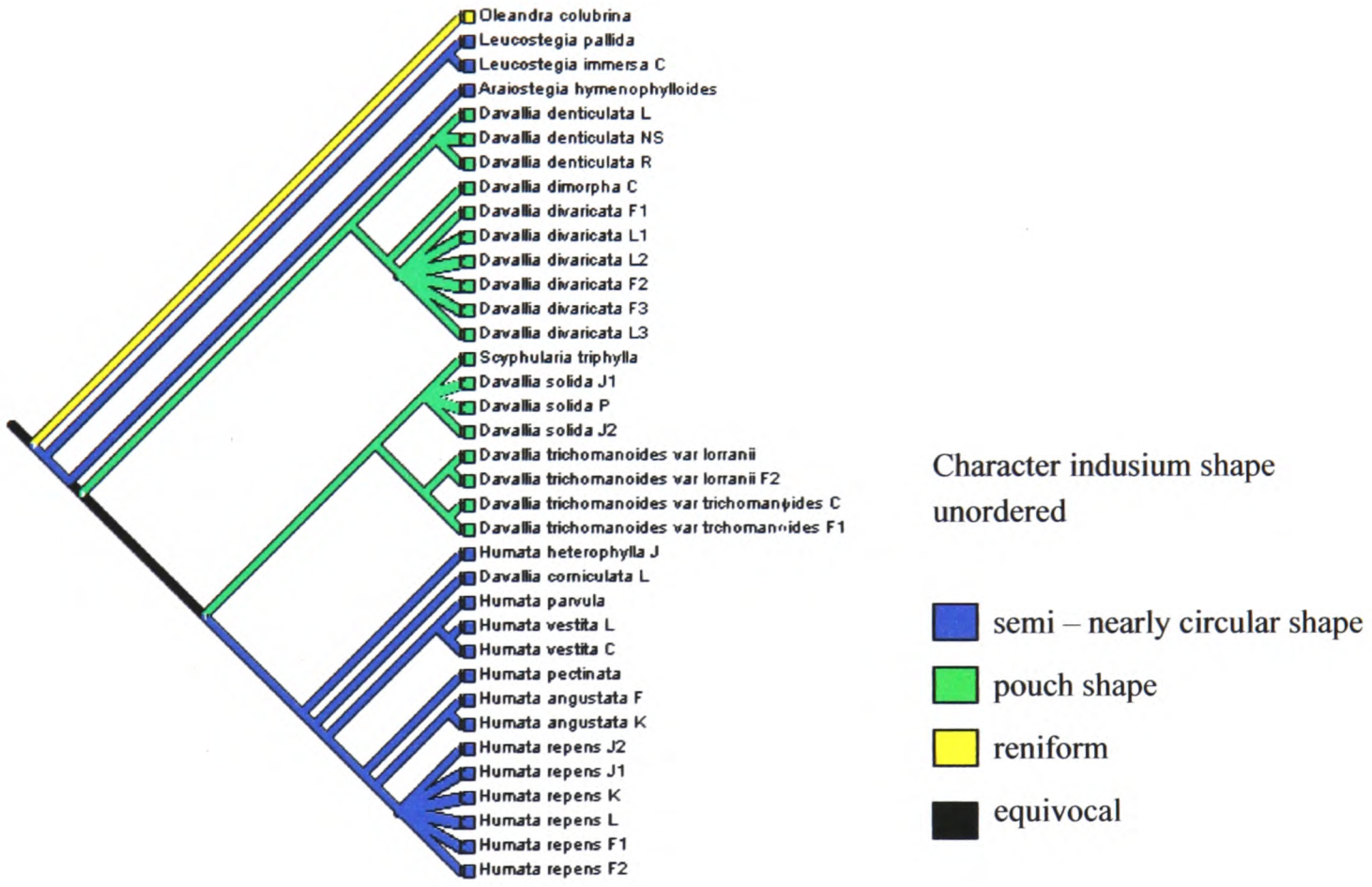


Figure 6.6: Inferred evolution of indusium shape via mapping the character onto the phylogenetic tree

6.4 **Discussion**

6.4.1 **Field observation**

Based on field observations, Davalliaceae in Peninsular Malaysia consists of four life forms which were true epiphytes (creeping on tree trunk or branches without connection with ground), lithophytes/epipetric (creeping on moist rock), hemi epiphytes (creeping on tree trunk and have connection with ground) and terrestrial plants. However for the purpose of scoring, only three life forms have been used: true epiphytes (including lithophytes), hemi epiphytes and terrestrial plants. Close affinity between lithophytes and epiphytes ferns exist because both sites required colonizers to have strong surface clinging ability.

The hemi epiphytes were further subdivided to primary hemi epiphytes and secondary hemi epiphytes. Primary hemi epiphytes are plants which initially grow as epiphyte and later becoming a terrestrial plant, whereas secondary hemi epiphytes begin as a terrestrial plant and then an epiphyte. Secondary hemi epiphytes are similar to climbers but only early in their lives do they depend on soil for water. It obtains water from the surface of the host plant, and/or directly from rain and mist or water from its own storage. Water storage can be an important adaptation to allow epiphytes to survive dry periods. In Davalliaceae, rhizome scales function to trap water from rain or mist, making it available to the plant.

Most species of Davalliaceae with large rhizomes (> 5mm) were true epiphytes. Large rhizomes enable better grip onto tree branches and provide adequate source of water storage. However *Humata* spp and *D. corniculata,* which have small rhizomes, were found as lithophytes and terrestrial plants. The smaller sized rhizomes easily detached from the host and fall onto the ground or on rock surfaces, becoming terrestrial or lithophytic plants. *Scyphularia triphylla* has discontinuous growth on trees. It was found on the basal and on the upper part of the tree trunk, but not in between. *Araiostegia hymenophylloides*, a basal clade in the phylogenetic tree, was mostly found as a lithophyte with a smaller proportion of individuals being terrestrial plants. When found as terrestrial plants it was usually growing close to individuals that grew as lithophytes, suggesting animal or wind factors for the dispersal of spores.

D. trichomanoides var. *lorrainii* with rough scale trichomes was found as an epiphyte whilst *D. trichomanoides* var. *trichomanoides* with finer scale trichomes was found as a terrestrial plant on slopes together with *D. corniculata. Humata vestita*, previously reported in only one locality (Cameron Highlands) was found creeping on the ground, on the side of the road close to the summit of Gunung Brinchang (Cameron Highlands).

6.4.2 **Morphological characters supporting clade division based on molecular phylogenetic tree**

a. **Araiostegia Clade**

This clade consists of a single species (*Araiostegia hymenophylloides*). This species was distinguished from all species of *Davallia* and *Humata* by several morphological characters, which were finely disserted lamina, thin lamina texture and location of sori at the bending point of a vein. In contrast, *Davallia* and *Humata* have sori at the forking point of a vein and a thick textured lamina.

b. **Davallia Clade I** (DCIa and DCIb)

This clade comprised three taxa: *D. denticulata, D. dimorpha* and *D. divaricata*. These species were linked together by the shared characters non acicular scale shape and scales with toothed margin. *D. dimorpha* and *D. divaricata* were grouped together (as DCIb) as they have dimorphic fronds, presence of false veins, pseudopeltate scale attachment and an herbaceous lamina. However the morphological characters that differentiate *D. dimorpha* from *D. divaricata* include narrow fertile frond, broader indusium and venation not reaching the margin in *D. dimorpha*.

c. **Davallia Clade II** (DCIIa and DCIIb)

Scyphularia triphylla, *D. solida*, *D. trichomanoides* var. *lorrainii and D. trichomanoides* var. *trichomanoides* were grouped together in this clade. *Scyphularia triphylla* formed a sister clade to *D. solida* (together they formed

Clade DCIIa), and so these species were more close to each other than to *D. trichomanoides*. Morphological characters supporting the relationship between *S. triphylla* and *D. solida* were black scales, thick rhizome (> 5mm), coriaceous lamina, venation reaching the margin and absence of false vein. *D. trichomanoides* (both varieties) were included in this clade with shared characters: black scales (except *D. trichomanoides* var. *trichomanoides*) and pinnate to quadripinnate frond (except *S. triphylla*).

d. **Humata Clade**

All *Humata* species plus *D. corniculata* were included in this clade. Species within this clade shared the morphological characters of small size rhizome, white waxy layer and basal attachment of indusium.

6.4.3 **Character evolution**

Character evolution in Davalliaceae generally exhibits homoplasy. Homoplasy is similarity that is not due to homology or common ancestory but the result of independent evolutionary change (Simpson, 2006). The presence of homoplasious characters contributed to difficulties in phylogenetic inference based on morphological characters, making genuine synapomorphic characters (those shared because of common ancestry) harder to identify. Only a few cases of apomorphic characters that evolved only once could be defined in this study. These apomorphies include white waxy rhizome which was unique to *Humata* clade, flattened rhizome found only in *Scyphularia triphylla* and basal attachment of scale and glabrous stipe in *Leucostegia*. However the Davallia

clade 1 could be recognized by a combination of characters, i.e. scale colour, structure of lamina and venation. This showed that morphological phylogenetic study by itself is an inadequate source of information to study evolutionary change.

6.4.4 **Evolution of epiphytes**

Epiphytism is a life habit that requires specialized vegetative and reproductive features. In ferns, these features include light and small size of spore, thin frond texture and long creeping rhizome with scales. Epiphytism in ferns has existed since palaeozoic times (Rothwell, 1991).

The most parsimonious tree with mapped life form characters (Figure 6.1) showed that epiphytism in Davalliaceae evolved from hemi epiphytes. This is in agreement with a previous study (Tsutsumi and Kato, 2006). The pattern of evolution of epiphytism can be determined, or understood, by correctly inferring family relationships. In Davalliaceae for example, phylogenetic study confirms *Oleandra* (Olendraceae) as sister clade to Davalliaceae (Schneider et al., 2004b), and suggesting that as *Oleandra* is epiphytic, the ancestor of *Davallia* was probably also epiphytic.

In *Trichomanes* (Hymenophyllaceae) Dubuisson et al. (2003) reported that epiphytes are derived from terrestrial ancestors or from hemi epiphytes, and that hemi epiphytes may also have been derived from a terrestrial ancestors. This contrasts with Aspleniaceae, where most *Asplenium* species are epiphytes

(including lithophytes); a previous study (Schneider et al., 2004a) supports *Hymenasplenium* (terrestrial) as the sister lineage to all other asplenioid. Therefore the epiphytic habit is considered to have evolved several times within leptosporangiate ferns.

6.5 **Conclusions**

The combination of molecular phylogeny and morphological characters provided a useful tool in unravelling the evolution events in Davalliaceae. Even though only three regions data was used for this study, several morphological characters supported the clades found. The mapping of the morphological characters onto molecular phylogenetic tree indicated that most of the characters evolved more than once. Most of the species in this study were true epiphytes and indicated that it was evolved from hemi epiphytes and the terrestrial habit has evolved numerous times within Davalliaceae. However morphological phylogenetic study in itself without additional information is insufficient to fully understand and confirm the evolutionary changes in Davalliaceae. Further work should emphasise finding additional informative characters from other disciplines, for instance anatomical characters.

Chapter Seven

General Discussion and Conclusion

7.1 The Paraphyly of *Davallia*

Advances of the theory and practice in cladistics, molecular techniques and computer technology provide systematists with more accountable tools for studying species variation and result in better understanding of plant relationships. Consequently, most of the earlier plant classifications need to be reviewed. This thesis looked at morphology, cytology and molecular (chloroplast region) data in an attempt to update the classification of Davalliaceae, especially in Peninsular Malaysia.

The results presented in this thesis showed moderate to strong support for paraphyly of *Davallia* especially in Peninsular Malaysia, and provided very good phylogenetic resolution within the genus, despite topological data based on the *trnL-F* region showing slight differences from that shown by other regions, or the combined analysis. The results were partially incongruent with the latest classification based on morphology (Nooteboom, 1998) but congruent with a preliminary study based on molecular data by Tsutsumi and Kato (2005). It also proved that *Leucostegia* should be separated from Davalliaceae as suggested by Tsutsumi and Kato (2005). The genus was tentatively placed in Dryopteridaceae (Smith et al., 2006; Schuettpelz and Pryer, 2007). Four major clades were recognised in Davalliaceae, namely Araiostegia clade (AC), four clades that comprise *Davallia*: Denticulata clade

(DCIa), Dimorpha-Divaricata clade (DCIb), Scyphularia-Solida clade (DCIIa), Trichomanoides clade (DCIIb), and lastly the Humata clade (HC).

Maximum parsimony and Bayesian analyses of *rps4-trnS* and combined three regions produced congruent topologies, but topologies of *rbcL* and *trnL-F* produced slight differences. Analysis of all 81 taxa (Figure 5.14) based on *rbcL* showed that all species were fully resolved within these clades without having to make a separate/regional lineage. Hence analyses of *trnL-F* and *rps4-trnS* might be expected to show the same result too.

Molecular phylogenies of Davalliaceae from Peninsular Malaysia based on both the *trnL-F* and *rps4-trnS* regions indicated that none of the genera (except *Scyphularia*) in this family is monophyletic. However the results produced could have been interpreted with more confidence if species from *Davallodes* and species from other parts of the world were included in the study.

Most genera of Davalliaceae in the study area were characterized by having a single base chromosome number, i.e. x = 40. All plants were recognised as diploid except *H. vestita* which was polyploid (hexaploid). Spores of Davallioid ferns were generally monolete (ellipsoid), with a thin layer of perispore and verruculate ornamentation (exospore). The spore size of the hexaploid *H. vestita* was larger (>80μm) compared with other diploid species (65 – 70 μm).

Phylogenetic reconstruction based on morphology was inconclusive and character evolution in the genus and related genera was characterised by homoplasy. However, the tree topology based on molecular data showed *Humata* spp (plus *D. corniculata*) to be monophyletic but nested within *Davallia*.

These results need to be considered from the viewpoint of old fashioned taxonomists and modern taxonomists. Taxonomists have tended to dispute findings of biologists that primarily depended on computer technologies, despite sharing similar goals to delimit the unit of life's diversity (Avise, 2000). Approaches based on morphological data alone have limitations as these exclusively discriminate on established morphological characters, and morphological characters are more prone to homoplasy and convergent evolution. Molecular data assist mainly in discovering cryptic species and to differentiate intraspecific and interspecific morphological variations, with limited application in other areas. An integrated approach is necessary to provide a holistic understanding of the species, and species relationships can be interpreted with more confidence with evidence from more than one area.

Given that the topology based on molecular data showed paraphyly in both genera, *Humata* should be placed within *Davallia*. This was suggested by Nooteboom (1998) because the morphological characters that support separation of *Humata* and *Davallia* were inconclusive and limited to the presence of white waxy rhizome, shell shape of indusium and attachment of

indusium. *Scyphularia*, nested inside the Davallia clade should be placed in *Davallia* too because only flattened rhizome and simple frond support the separation of *Scyphularia* from *Davallia*. *Araiostegia* should remain a distinct genus as topology based on molecular data showed it to be the basal clade within Davalliaceae, sister to that comprising all *Davallia* plus *Humata* and *Scyphularia*. *Leucostegia* should be excluded from Davalliaceae as the topology based on molecular data showed that this genus was closer to the outgroups. This was further supported by unique morphological characters in *Leucostegia*: rhizome with hair and scale (*L. immersa*), basifixed scale attachment, terminal location of sori, scaly indusium and glabrous stipe.

Based on these facts, Davalliaceae in Peninsular Malaysia consists of two genera: *Araiostegia* (one species) and *Davallia* (fourteen species). Complete details of each species are given in 'Davalliaceae of Peninsular Malaysia' (appendix I)

7.2 **Taxonomic discussion**

My estimate of phylogeny based on chloroplast DNA sequences did not agree with many traditional generic delimitations in Davalliaceae. However the recent revision by Nooteboom for Flora Malesiana was partially consistent with the phylogeny presented here.

7.2.1 **Family delimitation**

The phylogeny showed *Oleandra colubrina* (Oleandraceae) as sister to all members examined. However, *Leucostegia* (also Davalliaceae) was also sister to this wider group, implying paraphyly in genera of Davalliaceae. This was similar to Tsutsumi and Kato's (2005) findings, which indicated that *Leucostegia* should be excluded from Davalliaceae.

7.2.2. **Generic delimitation**

A. *Araiostegia*

Araiostegia hymenophylloides was sister to the rest of Davalliaceae. Although a few species of *Araiostegia* nested in the *Davallia* clade according to *rbcL* data (Figure 5.14), I disagree with Nooteboom (1998) and consider that it should be recognized as a separate genus. *Araiostegia* is morphologically distinct by its finely disserted lamina, thin lamina texture, entire scales margin, venation not reaching the margin, sori located at bending point of vein and scaly indusium.

B. *Scyphularia*

Scyphularia triphylla was nested inside the broader Davallia clade, which argues against its position as a separate genus. Based on criteria of monophyly, Nooteboom treated *Scyphularia triphylla* as *Davallia triphylla* with two other former members of *Scyphularia* under *Davallia* section *Scyphularia*. The remainder of *Davallia* (including *Humata*) was treated as *Davallia* section *Davallia*, but this group was not monophyletic in my phylogeny.

C. *Humata-Davallia*

In this phylogeny, *Humata* is monophyletic only if:

1. Either *Humata heterophylla* or *Davallia corniculata* are excluded from the phylogeny, or
2. *D. corniculata* is treated as *Humata corniculata*
3. *H. heterophylla* is treated as *Davallia heterophylla*

However, even if either of the latter two arrangements were accepted, *Humata* would still be strongly supported as nested inside a wider *Davallia*. This would make *Davallia* paraphyletic. Nooteboom (1998) did not accept *Humata* as distinct from *Davallia*, and his treatment agrees with the phylogeny presented here, suggesting a broad and monophyletic definition of *Davallia* to include *Humata* and *Scyphularia*.

7.2.3. **Species delimitation**

A few taxa of Davalliaceae which were treated as different species in Peninsular Malaysia in older classifications were treated as a varieties or synonyms in Nooteboom's (1998) classification:

Davallia dimorpha, an endemic species (Holttum, 1968), was treated as *D. divaricata* var. *dimorpha* but the phylogenetic results in this study (Figure 5.14) show that *D. divaricata* from Peninsular Malaysia was linked together with *D. formosana* from Taiwan and *D. dimorpha* was linked with *D. divaricata* from Indonesia.

Humata vestita was treated as a synonym of *D. repens* (*Humata repens*). My phylogeny indicated that *H. vestita* (Peninsular Malaysia) was a sister clade to *H. parvula* while *H. repens* was sister clade to *H. angustata* and *H. vestita* (Java).

Davallia trichomanoides consists of two varieties: *D. trichomanoides* var. *trichomanoides* and *D. trichomanoides* var. *lorrainii*. The difference between these two taxa was only on scale colour and the trichomes on scale margin but the phylogeny showed that *D. trichomanoides* var. *trichomanoides* was sister clade to *D. trichomanoides* while *D. trichomanoides* var. *lorrainii* was sister clade to *Davallia petelotii* (Laos). It was also found that the two varieties are different ecologically and geographically. *D. trichomanoides* var. *trichomanoides* was found as terrestrial plants meanwhile *D. trichomanoides* var. *lorrainii* as an epiphytes. Based on this var. *lorrainii* should be raised to species level.

7.2.4. **Sectional classification in *Davallia* sensu Nooteboom**

Nooteboom treated a broad and inclusive *Davallia,* using two sections: *Davallia* section *Davallia* to include all *Davallia* sensu lato plus all *Humata* species and *Davallia* section *Scyphularia* which include only the members of the former genus *Scyphularia*. If *Davallia* were treated in this broad sense, the three major clades resolved in this phylogeny (DCI, DCII and HC in Figure 6.1) offer themselves as more useful sections than those of Nooteboom.

I propose that *Scyphularia* and *Humata* be included in *Davallia* (in agreement with Nooteboom, 1998) and for Peninsular Malaysian taxa, it appeared that the three sections which correspond to three clades could provide a meaningful valuable, functional monophyletic subgeneric groups.

Formal taxonomic decision on this cannot be made until the following are known:

- Phylogenetic placement of the type species *D. canariensis* Sm. as this would determine which section became *Davallia* section *Davallia*.
- Phylogenetic placement of *Humata ophioglossoides* Cav. (type species of *Humata*) would determine if *Davallia* section *Humata* was a valid name for the Humata clade.
- The placement of other species not examined so far. Inclusion of further species from the genus is necessary before the monophyly of these groups can be assured. In particular *Davallodes* and other species of *Araiostegia* must be included in future studies.

The revision of Peninsular Malaysian species in appendix I was based on these putative sections with the following names:

1. *Araiostegia hymenophylloides*

2. *Davallia* spp. in three sections:

    a. *Davallia* section *Davallia* (DCI on Figure 6.1)

    The species included in this section were *D. denticulata*, *D. dimorpha* and *D. divaricata.*

    b. *Davallia* section *Scyphularia* (DCII on Figure 6.1)

    The species included in this section were *Scyphularia triphylla*, *D. solida* and both varieties of *D. trichomanoides*

    c. *Davallia* section *Humata* (HC on Figure 6.1)

    All *Humata* species plus *D. corniculata* included in this section.

7.3 **Future research**

Future work should concentrate on wider sampling of taxa and adding more morphological characters. Although molecular results from this study and previous study confirmed the paraphyly of genera in Davalliaceae, further molecular work based on other variable regions (such as *matK*) and the nuclear genome (e.g. ITS), might improve the resolution of relationships within Davalliaceae. Future field work should concentrate on broader sampling and sequencing of all species within the family and closely related species. In general, the combination of morphological and molecular data in constructing a phylogenetic tree worked well to address problems in relationship within Davalliaceae and contribute to a new approach for classification of the genus. However, further taxonomic and nomenclatural work as mentioned above is needed to solve the problem in classification.

REFERENCES

Abraham, A., Ninan, C.A. and Matthew, P.M. 1962. Studies on the cytology and Phylogeny of the pteridophytes. VII. Observations on one hundred species of S. Indian Ferns. *J. Ind. Bot. Soc.* **XLI.** (3):339-421.

Alfaro M.E., Zoller S. and Lutzoni, F. 2003. Bayes or bootstrap? A simultation study comparing th performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Mol. Bio. Evol.* **20**(2): 255–266.

Alston, A. H. G. 1956. The subdivision of the Polypodiaceae. *Taxon* **5**:23-25.

Atkinson, L.R. 1973. The gametophyte and family relationships. In Jermy, A.C., Crabbe, J.A. and Thomas, B.A.. The phylogeny and classification of the ferns. *Bot. J. Linn. Soc.* vol **67**.supp. 1.73-90

Avise, J.C. 2000. *Phylogeography: the history and formation of species.* Cambridge:Harvard Uni. Press.

Benzing, D.H. 1987. Vascular epiphytism: Taxonomy participation and adaptive diversity. *Ann. Miss. Bot. Gard.* **74(2)**:183-204.

Benzing, D.H. 1989. The evolution of epiphytism. In Luttge, U. (ed.) *Vascular plant as epiphytes.* Berlin, springer.15-41.

Bidin, A. 1984. A chromosome count for the fern genus *Osmunda* in P. Malaysia. *Fern Gaz.* **12**(6):360-362.

Bidin, A. 1995. The ecology and cytology of *Brainea insignis* (HK.) J. Sm. (Blechnaceae). *Fern Gaz.* **15**(1):21-24.

Bidin, A. 2000. Contribution to the cytology and taxonomy of the ferns of Malaysia. *Mal. Nat. J.* **54**(4):289-304.

Bidin, A and Go, R. 1995. The chromosome base numbers for *Tectaria* and its allied genera in P. Malaysia. *Nat. Hist. Res. Chiba* **4**(1):49-56.

Bidin, A. and Walker, T. G. 1994. The distribution of *Adiantum caudatum* cytotypes in South East Asia. *Mal. Nat. J.* **48**:79-82

Bradley, M. V. 1948. A method for making aceto carmine squashes permanent without removal of coverslip. *Stain Tech.***12**:123-124.

Bremer K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795–803.

Bremer, B., Jansen, R.K., Oxelmen, B., Backlund, M., Lantz, H. and Kim, K.J. 1999. More characters or more taxa for a robust phylogeny - case study from the coffee family (Rubiaceae). *Systematic Biology* **48**(3): 413–435.

Brummitt, R.K. 1992. *Vascular plant families and genera*. Kew.

Cavanilles, A.J. 1802. *Descripcion de las plantas*: 272, 273, 278, 279, 679.

Ching, R.C. 1940. On natural classification of the family "Polypodiaceae" *Sunyatsenia* **5**(4): 201-268.

Christensen, C. 1938. Filicinae. In F. Verdoorn (ed.). *Manual of Pteridology*. Pp 522-550. The Hague: Nijhoff.

Clark, A. and Hollingsworth, M. 2006. *Molecular biology for phylogenetic and population genetic studies. Vol. I and II.*- Molecular laboratory manual. Royal Botanic Garden, Edinburgh.

Copeland, E.B. 1908. New or interesting Philippine ferns:111. *Philip. J. Sci.* **3**(1):31-45.

Copeland, E. B. 1927 *Davallodes* and related genera. *Philip. J. Sci.* **34**(3):229-257.

Copeland, E.B. 1947. *Genera Filicum*. The Genera of ferns (Chronica Botanica, Annales Cryptogamici et Phytopathologici no. 5): xiv + 247 pp.

Crabbe, J.A and Jermy, A.C. 1975. A new generic sequence for the pteridophyte herbarium. *Fern Gaz.* **11**:141-161.

Cranfill, R. 2000. Phylogenetic utility of plastid ribosomal protein S4(rps4) in land plants. *Amer. J. Bot.* **87**(suppl.):121.

Crawford D.J. 1990. *Plant Molecular Systematics-Macromolecular approaches*. John Wiley and Sons. New York.

de Joncheere, G. J. 1977. Specific concept in *Humata pectinata* (J.E. Smith) Desv. *Gard. Bull. Sing.* **30**: 45-58.

Dickason, F.G. 1946. The ferns of Burma. *Ohio J. Sci.* **46**: 109-141.

Diels, L. 1902. Polypodiaceae. In Engler, A and K. Prantl. *Die Naturlichen Pflanzenfamillien*, Liepzig. **1,** 4: 204-222

Doyle, J.J. and Doyle, J.L. 1990. Isolation of plants DNA from fresh tissues. *Focus* **12**: 13-15.

Dubuisson J-Y, Hennequin, S., Rakotondrainibe, F., and Schneider, H. 2003. Ecological diversity and adaptive tendencies in the tropical fern *Trichomanes* L. (Hymenophyllaceae) with special reference to climbing and epiphytic habits. *Bot. J. Linn. Soc.* **142**:41-63.

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

Ebihara, A., Iwatsuki, K., Kurita, S. and Ito, M. 2002. Systematic position of *Hymenophyllum rolandi-principis* Rosenst. or a monotypic genus *Rosenstockia* Copel. (Hymenophyllaceae) endemic to New Caledonia. *Acta Phytotax. Geobot.* **53**(1):35-49.

Eriksson T. 1999. *AutoDecay version 4.0.* Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm.

Farris, J.D., Kallersjo, M., Kluge A.G. and Bult, C. 1994. Testing the significance of incongruence. *Cladistics* **10**:315-319.

Fée, A.L.A. 1852. Memoires sur les familles des fougeres. 5. *Genera filicum*. Polypodiacees: 319-533.

Felsenstein J. 1983. Parsimony in systematics: Biological and statistical issues. *Ann. Rev. Eco. Syst.* **14**: 313–333.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**:783-791.

Frank, A.B. 1877. Davalliaceae. In Leunis: Syn. Pflanzenk. Ed. 2,3: 1474.

Gastony, G.J. and Rollo, D. R. 1995. Phylogeny and generic circumscriptions of cheilanthoid ferns (Pteridaceae: Cheilanthoideae) inferred from *rbcL* nucleotide sequences. *Amer. Fern J.* **85**: 341-360.

Grant, V. 1981. *Plant Speciation*. 2nd ed. Columbia uni. Press, NY

Hall B.G. 2004. *Phylogenetic trees made easy. A how-to manual.* 2nd edition. Sinauer Associates Inc.. Sunderland, Massachusetts, U.S.A.

Hasebe, M., and Iwatsuki, K.1990. Chloroplast DNA from *Adiantum capillus-veneris* L., a fern species (Adiantaceae); clone bank, physical map, and unusual gene localization in comparison with angiosperm chloroplast DNA. *Curr. Genet.* **17:** 359-364.

Hasebe, M., Wolf, P.G., Pryer, K.M., Ueda, K., Ito, M., Sano, R., Gastony, G.J., Yokoyama, J., Manhart, J.R., Murakami, N., Crane, E.H., Haufler, C.H. and Hauk, W.D. 1995. Fern phylogeny based on *rbcL* nucleotide sequences. *Amer. Fern J.* **85**: 134-181.

Haufler, C. 1995. Species concepts and speciation in pteridophytes. In Camus et al. *Pteridology in perspective*: proceedings of the Holttum memorial pteridophyte symposium:291-305.

Hauk, W.D. 1995. A molecular assessment of relationships among cryptic species of *Botrychium* subgenus Botrychium (Ophioglossaceae). *Amer. Fern J.* **85**: 375-394.

Hauk, W.D., Parks, C.R. and Chase, M.W. 2003. Phylogenetic studies of Ophioglossaceae: evidence from *rbcL* and *trnL-F* plastid DNA sequences and morphology . *Mol. Phy. Evo.* **28**(1):131-151.

Hill, C.R. and Camus, J.M. 1986. Evolutionary cladistics of marattialen ferns. *Bull. Brit. Mus. (Nat. Hist.) Bot.* **14**:219-300

Holttum, R.E. 1938. The ecology of tropical pteridophytes. In Verdoorn Fr, (ed.). *Manual of pteridology*. Leiden, Martinus Nijhoff, 420-450.

Holttum, R.E. 1947. A revised classification of the Leptosporangiate ferns. *J. Linn. Soc. Bot.* **53**:123-158.

Holttum, R. E. 1949. The classification of ferns. *Biol. Rev.* **24**:267-296.

Holttum, R.E. 1968. *A revised flora of Malaya, an illustrated systematic account of Malaya flora, including commonly cultivated plant, vol 2. Ferns of Malaya*, 2nd edn. Singapore government printing.

Holttum, R.E. 1972. The genus *Davallodes*. *Kew Bull.* **27**(2):245-249

Ingrouille, M. and Eddie, B. 2006. Plants: *Evolution and Diversity*. Uni. Cambridge Press.

John, R. J. 1995. Malesia-An introduction. *Curtis's Bot. Mag.* Kew **12**(2):52-62.

Jong, K. 1997. *Laboratory manual of plant cytological techniques*. Royal Botanic Garden Edinburgh, Edinburgh.

Judd, W.S., Campbell, C.S., Kellogg, A.E. and Stevens, P.F. 1999. *Plant systematic: A phylogenetic approach*. Sinauer Ass. Inc.

Jukes, T.H. and Cantor, C.R. 1969. Evolution of protein molecules. In Munro, H. N. (ed.) *Mammalian Protein Metabolism III*, Academic Press, New York.

Kato, M. 1975. Taxonomic note on the cordate scales found in *Davallia*. *Acta Phtyotax. Geobot.* **26**(5-6): 152-159.

Kato, M. 1985. A systematic study of the genera od the fern family Davalliaceae. *J. Fac. Sci.* Univ. Tokyo. III. **13**:553-5573.

Kato, M. and Mitsuta, S. 1980. Stelar organization in davallioid ferns. *Phytomorphology* **29**: 362-369.

Kimura, M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. *Proceeding of the National Academy of Sciences of the United States of America.* (Part 2:Biological Sciences.) 78(1): 454-458.

Kluge, A. G. and Wolf, A. J. 1993. Cladistic: what's in a word? *Cladistics* **9**: 183-199.

Kramer, K.U. and Green, P.S. 1990. Pteridophytes and Gymnosperms Vol. I. in Kubitzki, K. (ed.) *The Families and Genera of Vascular Plants.* Heidelberg: Springer-Verlag.

Kramer, K.U. 1990. Davalliaceae. in Kubitzki, K. (ed.) *The Families and Genera of Vascular Plants.* Vol. I. Pteridophytes and Gymnosperms, Heidelberg: Springer-Verlag. 52-57.

Kramer, K.U. and Tryon, R.M. 1990. Introduction to the treatment of pteridophytes. In K. Kubitzki (ed.) *The Families and Genera of Vascular Plants*. Vol 1. Heidelberg: Springer-Verlag. 12-13.

Little, D.P. and Barrington, D. S. 2003. Major evolutionary event in the origin and diversification of the fern genus *Polystichum* (Dryopteridaceae). *Amer. J. Bot.* **90**:508-514.

Lovis, J.D. 1977. Evolutionary patterns and processes in ferns. *Adv. Bot. Res.* **4**:229-415.

Maddison W.P. and Maddison, D.R. 2003. *MacClade 4. Analysis of Phylogeny and Character Evolution.* Version 4.06 OS X. Sinauer, Sunderland, Massachusetts.

Manton, I. 1950. *Problems of cytology and evolution in Pteridophyta.* Cambridge Univ. Press. London.

Manton, I. 1968. Cytological notes on 100 species of Malayan ferns. In Holttum, R.E. 1968. *The flora of Malaya* vol. **2**. *Ferns*. Singapore.

Manton, I and Sledge, W.A 1954. Observation on the cytology and taxonomy of the Pteridophyte flora of Ceylon. *Phil. Trans. Roy. Soc. London. Ser. B.* **238**:127-185.

Manton, I., Lovis, J.D., Vida, G. and Gibby, M. 1986. Cytology of the fern flora of Madeira. *Bull.of the British Museum (Natural History) Botany.* Series **15**:123-161.

Mettenius, G.H. 1856. *Filices Horti Botanical Lipsiensis*. Leipzig: Voss.

Moore, D.M. 1978. The chromosome and plant taxonomy. In Street, H.E. *Essays in Plant taxonomy*. Academic press.

Moran, R.C. 1982. The *Asplenium trichomanes* complex in the United States and adjacent Canada. *Amer. Fern J.l* **72**: 5–11.

Nadot, S., Bajon,R. and Lejeune, B. 1994. The chloroplast gene rps4 as a tool for the study of Poaceae phylogeny. *Pl. Syst. Evol.* **191** : 27-38.

Nayar, B.K. and Bajpai, N. 1977. Morphology in relation to phylogeny of the Davallioid-Oleandroid group of ferns. *Phytomorphology* **26**:333-354.

Nieder, J., Engwald, S. and Barthlott, W. 1999. Patterns of neotropical epiphyte diversity. *Selbyana* **20**(1):66-75.

Nooteboom, H.P. 1992. Notes on Davalliaceae I. The genera *Araiostegia, Davallodes, Leucostegia* and *Gymnogrammitis*. *Blumea* **37**(1):165-187.

Nooteboom, H.P. 1994. Notes on Davalliaceae II. A revision of the genus *Davallia*. *Blumea* **39**(1-2):151-214.

Nooteboom, H.P. 1998. Davalliaceae. *Flora Malesiana.* Series II- ferns and fern allies. **3**:235-276.

Parris, B.S. and Latiff, A. 1997. Towards a pteridophyte flora of Malaysia: A provisional checklist of taxa. *Mal. Nat. J.*. **50**: 235-280

Pennington R.T. 2000. Introduction. In Scotland R. and Pennington, R.T. (eds). *Homology and Systematics. Coding charaters for phylogenetic analysis.* The Systematics Association Special Volume. Series 58. Taylor and Francis, London, New York.

Pérez Arbeláez, E. 1928. Die natürliche gruppe der davalliaceen (Sm.) Kfs. *Bot. Abh. (Jena)***14**: 1-96.

Philip, D. A. and White, R.A. 1967. Frond articulation in species of Polypodiaceae and Davalliaceae. *Amer. Fern J.* **57**:78-88.

Pichi-Sermolli, R.E.G. 1977. Tentamen pteridophytorum genera in taxonomicum ordinem redigendi. *Webbia.* **31**(2):313-512.

Pichi-Sermolli, R.E.G .1959. Pteridophyta. In W.B. Turrill (ed.) *Vistas in botany*. London, Pergamon Press.

Pintér, I., Bakker, F.T., Barrett, J.A., Cox, C., Gibby, M., Henderson, S., Morgan-Richards, M., Rumsey, F.J., Russell, S.J., Schneider, H., Trewick, S.A. and Vogel, J.C. 2002. Phylogenetic and biosystematic relationships in four highly disjunct polyploid complexes in the subgenera *Ceterach* and *Phyllitis* in *Asplenium* (Aspleniaceae). *Organisms, Diversity and Evolution* **2**:299-311.

Posada, D. and Buckley, T.R. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over Likelihood Ratio Tests. *Syst. Bio.* **53**(5): 793–808.

Posada, D. and Crandall, K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 490–500.

Posada, D. and Crandall, K.A. 2001. Selecting the best-fit model of nucleotide substitutions. *Syst. Bio.* **50**(4): 580–601.

Presl, K.B. 1836. *Tentamen Pteridograph.* Prague:Haase.iae

Pryer, K.M. 1999. Phylogeny of marsileaceous ferns and relationships of the fossil *Hydropteris pinnata* reconsidered. *Int. J. Pl. Sci.* **160**:931-954.

Pryer, K.M., Schuettpelz, E., Wolf, P.G., Schneider, H., Smith, A.R. and Cranfill, R. 2004. Phylogeny and evolution of ferns (monilophytes) with focus on the early leptosporangiate divergences. *Amer. J. Bot.* **91**:1582-1598.

Pryer, K.M., Smith, A.R., Skog, J.E. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. *Amer. Fern J.* **85**:202-282

Richardson, J.E., Fay, M.F.,Croonk, Q.C.B., Bowman, D. and Chase, M.W. 2000. A Phylogenetic analysis of Rhamnaceae using *rbcL* and *trnL-F* plastid DNA sequences. *Amer. J. Bot.* **87**(9):1309-1324.

Richardson, P.M. 1983. C-Glycosylxanthones in the fern genera *Davallia, Humata* and *Nephrolepis. Phytochemistry* **22** (1): 309-311.

Rödl-Linder, G. and Nooteboom, H.P. 1997. Notes on Davalliaceae III. In Johns, R.J. (ed.): *Holttum memorial volume*, Royal Botanical Garden, Kew: 67-79.

Rothwell, G.W. 1991. *Botryopteris forensic* (Botryopteridaceae), a trunk epiphyte of the tree fern *Psaronius*. *Amer. J. Bot.* **78**:782-788.

Schneider, H., Smith, A.R., Cranfill, R., Haufler, C.H., Ranker, T.A. and Hildebrand, T. 2002. *Gymnogrammitis dareiformis* is a polygrammoid fern (Polypodiaceae)- Resolving an apparent conflict between morphological and molecular data. *Plant. Syst. Evol.* **234**: 121-136.

Schneider, H., Russell, S.J., Cox, C.J., Bakker, F., Henderson, S., Rumsey, F., Barrett, J., Gibby, M. and Vogel., J.C. 2004a. Chloroplast phylogeny of Asplenioid ferns based on *rbcL* and *trnL-F* spacer sequences (Polypodiidae, Aspleniaceae) and its implications for biogeography. *Syst. Bot.* **29**(2):260-274.

Schneider, H., Smith, A.R., Cranfill,R., Hilderbrand, T., Haufler, C.H. and Ranker, T.A. 2004b. Unreaveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): exploring aspects of the diversification of epiphytic plants. *Mol. Phy. Evol.* **31**(3): 1041-1063.

Schuettpelz, E. and Pryer, K.M. 2007. Fern phylogeny inferred from 400 leptosprangiate and three plastid genes. *Taxon* **56**(4):1037-1050.

Scotland, R.W., Olmstead, R.G. and Bennett, J.R. 2003. Phylogeny reconstruction: The role of morphology. *Syst. Bio.* **52**(4): 539–548.

Sen, T. and Sen,U. 1970. A comparative study of the morphology and anatomy of *Leucostegia* and *Araiostegia*. *New Phytol.* **69**:1093-1101.

Sen, T. 1992. On the structure of spore of Davallioid genera: *Leucostegia* Presl, *Araiostegia* Copel. and *Scyphularia* Fee. *Ind. Fern J.* **9**:125-132

Sen, T., Sen, U. and Holttum, R.E. 1971. Morphology and anatomy of the genera *Davallia*, *Araiostegia*, *Davallodes* with a discussion on their affinities. *Kew. Bull.* **27**(2):217-243.

Simmons M. P. and Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst. Bio.* **49**(2): 369–381.

Simmons M. P., Ochoterena, H. and Carr, T.G. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. *Syst. Bio.* **50**(3): 454–462.

Simpson, M.G. 2006. *Plant systematics*. Elsevier academic press. UK.

Singh, G. 2004. *Plant systematics: An integrated approach.* Science Pub. Enfield.

Singh, V.P. and Roy, S.K. 1988. Cytology of forty species from Sikkim, Himalaya. *Ind. Fern J.* **5**:162-169.

Skog, J. E., Mickel, J. T., Moran, R. C., Volovsek, M. and Zimmer, E. A. 2004. Molecular studies of representative species in the genus *Elaphoglossum* (Dryopteridaceae) based on cpDNA sequences *rbcL*, *trnL-F* and *rps4-trnS. Int. J. Pl. Sci.* **165**:1063-1075.

Skog, J. E., Zimmer, E and Mickel, J. T. 2002. Additional support for two subgenera of *Anemia* (Schizaeaceae) from data for the chloroplast intergenic spacer region *trnL-F* and morphology. *Amer. Fern J.* **92**:119-130.

Smith, A. R and Cranfill, R. B. 2002. Intrafamilial relationships of the thelypteroid ferns (Thelypteridaceae). *Amer. Fern J.* **92**(2):131-149.

Smith, A.R., Pryer, K.M., Schuettpelz, E., Korall, P., Schneider, H. and Wolf, P.G. 2006. A classification for extant ferns. *Taxon* **55**(3):705-731.

Smith, J. 1842. An arranggement and definition of the genera of ferns with observation on the affinities of each genus. *Lond. J. Bot.* **1**:419-438, 659-668.

Smith, J. E. 1793. Tentamen Botanicum de Filicum generibus dorsiferarum. *Mem. Accad. Sci. Turin* **5**: 414-415.

Stace, C. A. 1989. *Plant taxonomy and biosystematic.* 2nd ed. Edward Arnold, London

Stebbins, G.L. 1950. *Variation and evolution in plants*. New York. Columbia Univ. Press.

Stein, D. B. 1985. Nucleic acid comparison as a tool in understanding species interrelationships and phylogeny. *Proc. Royal Soc. Edinb.* , **86B**:283-288

Swofford D. L. 2002. *PAUP: Phylogenetic analysis using parsimony (and other methods).* Version 4.0b10. Sinauer, Sunderland, Massachusetts.

Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. 1991. Universal primer for amplification of three non coding regions of chloroplast DNA. *Plant Mol. Bio.* **17**: 1105-1109.

Tavaré, S. 1986. Some probabilistic and statistical problems on the analysis of DNA sequences. In: *Lectures in mathematics in the life sciences*, vol **17**:57–86

Trewick, S. A., Morgan-Richards, M., Russell, S.J., Henderson, S., Rumsey, F.J., Pintér, I., Barrett, J.A., Gibby, M. and Vogel, J.C. 2002. Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. *Mol. Eco.* **11**:2003-2012.

Tryon, A.F. and Lugardon, B. 1991. *Spore of the Pteridophytes*. Springer-Verlag., New York.

Tsutsumi, C. and Kato, M. 2005. Molecular phylogenetic study on Davalliaceae. *Fern Gaz.* **17**(2):143-158.

Tsutsumi,C and Kato, M. 2006. Evolution of epiphytes in Davalliaceae and related ferns. *Bot. J. Linn. Soc.* **151**:495-510.

Vida, G. 1972. Cytotaxonomy and genome analysis of the European ferns. In Vida, G. (ed.) *Evolution in plants*. Symposia Biologica Hungarica. Akademiai, Budapest.

Walker, T.G. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. *Trans. R. Soc. Edinb.*, **66**:169-237

Walker, T.G. 1979. The cytogenetics of ferns. In Dyer, A.F. *The experimental biology of ferns*. Academic press.

Walker, T.G. and Jermy, A.C. 1982. Ecology and cytology of *Phanesorus* (Matoniaceae). *Fern Gaz.* **12**:209-213.

Whitten , W.M., Chase, M.W. and Stern, W.L. 1996. Molecular systematics of Stanhopeinae (Orchidaceae). *Amer. J. Bot.* **83** (suppl.):201.

Wiens, J.J. 2004. Speciation and ecology revisited: phylogenetic niche conservation and the origin of species. *Evolution* **58**:193-197.

Wilcox, T.P., Zwickl, D.J., Heath, T.A. and Hillis, D.M. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Mol. Phy. Evol.* **25**(2)361-371.

Notes on Davalliaceae of Peninsular Malaysia

DAVALLIACEAE

Davalliaceae Mett. ex Frank in Leunis, Syn. Pflanzenkd., ed. 2,3 (1877) 1747; K. U. Kramer in K. Kubitzki (ed.), Fam. & Gen. Vasc. Plnt. 1 (1990) 74-80; Kato, J. Fac. Sci. Univ. Tokyo. Sect. 3 (13) (1985) 553-573; Noot., Fl. Mal. Ser II (3) (1998) 235-276.

Epiphytic, epilithic or terrestrial. Rhizome dorsiventral, creeping and covered with scales. Leaves alternate in two ranks on the dorsal side of the rhizome. Roots on the ventral side of lateral buds of the rhizome. In *Araiostegia* and a few species of *Davallia* the scales with a cordate base, overlapping, attached (sometimes called pseudopeltate). In the other species the rhizome scales are peltate. The scales acicular, flat and nearly acicular, evenly narrowed towards the apex above the much broader base, or just evenly narrowed. In a number of species apical and marginal multicellular hairs on the scales. Scales often ciliate or toothed. Incision of the leaves very diverse, from simple entire frond to bipinnate to quadripinnate frond with uni-veined ultimate segments. Axes adaxially grooved, the grooves with raised centre; costae and costules adaxially convex. Lamina often firm in texture, herbaceous or coriaceous usually triangular, sometimes narrowed towards the base. Veins pinnately branched, free, reaching margin or sometimes not. False veins, sometimes present between the true veins in a few species. Sori at the bending point or at the forking point of veins, and the indusium attached at the base, at the sides or partially; semi circular or pouch shaped.

Distribution

Throughout the mainland from north (Perlis) to south (Johore) including offshore islands.

Chromosomes

Chromosome counts indicate that all davallioid ferns in Peninsular Malaysia are diploid (n = 40) except *Davallia vestita* collected from Cameron Highlands, Pahang which is hexaploid (n = 120).

Key to the genera/species

1a. Lamina compound, finely dissected, thin, sori located at bending point of vein..... *1. Araiostegia hymenophylloides*

1b. Lamina simple to compound, thin or coriaceous, sori at forking point of vein..... 2. *Davallia*

1. ***Araiostegia*** Copel.

Rhizome creeping, densely covered with scales; Scales always with cordate base overlapping (pseudopeltate); stipes articulated to the rhizome. Lamina deltoid, finely dissected, thin texture. Sori at bending point of vein; indusium small, semi-circular, attached by the base only.

1. ***Araiostegia hymenophylloides*** (Blume) Copel.

Araiostegia hymenophylloides (Blume) Copel., Philip. Journ. Sci. 34 (1927) 241; Holttum, Rev. Fl. Malaya, ed. 2, 2 (1968) 363; Noot., Blumea 37 (1992) 171.- Type: Blume s.n. (L sh 909.30-144), Java, Mt Burangran.
- *Aspidium hymenophylloides* Blume, Enum. Pl. Java (1828) 172; Noot., Fl. Mal. Ser. 2. 3 (1998) 255; - *Leucostegia hymenophylloides* (Blume)Bedd., Ferns S. India (1863) t. 252; - *Davallia hymenophylloides* (Blume) Kuhn, Noot., Fl. Mal. 2, 3 (1998) 255.
- *Cystopteris dalhousiana* Fée, Mém. Foug. 8 (1857) 108. – Type: Dalhousie in Herb. Delessert (G holo; K), Penang.

Rhizome 3-20 mm thick. Scales brown, narrowed evenly towards the apex, not bearing multiseptate hairs, lacking marginal setae or teeth, pseudopeltate with cordate base and with overlapping lobes. Stipes dark brown, adaxially grooved, 15-40 cm long, glabrous or with few scales. Fronds compound, tripinnate, elongate, often narrowing towards base, glabrous, 20-60 cm long by 10-40 cm broad, not or slightly dimorphic. Veins in sterile fronds frequently simple, not reaching the margin. False veins not present. Sori separate, frequently single on a segment at the bending point of a vein. Indusium reniform, attached at the narrow cordate base only, wider than long.

Distributions: Negeri Sembilan, Pahang, Penang and Perak.

Ecology: Epiphytic or epilithic, rarely terrestrial. Altitude 500-2200 m.

Specimens examined

Negeri Sembilan, Ulu Pedas Md Nur,11722 [K].

Pahang, Cameron Highland, Robinson's fall, Zulkifli & Rusea ZM 133 [UKMB];AG Piggott 3033, 3083 [UKMB]; Fraser Hills, near Gap, Parris 10579 [K]; GH Addison 37204 [SING].

Penang, Dr Kings collector 1309 [SING]; HN Ridley 7138 [K].

Perak, Bukit Larut, H Maideen and R Jaman HM6035 [UKMB]; Maxwell Hill (Bukit Larut), post off., J Sinclair 6171 [SING]; Taiping Hill, FEW Venning M 166 [K].

2. ***Davallia*** Sm.

Davallia Sm., Mém. Acad. Sci. Turin 5 (1793) 414; Copel., Fern Fl. Philip. (1958) 170; Holttum, Rev. Fl. Malaya, (1968) 354; Noot., Blumea 39 (1994) 155. - Type species: *Davallia canariensis* (L.) Sm.

Humata Cav., Descr. PI. (1802) 272; Copel., Fern Fl. Philip. (1950) 175; Holttum, Rev. Fl. Malaya, (1968) 364. - Type species: *Humata ophioglossa* Cav.

Wibelia Bernh. (non Fee 1852), J. Bot. (Schrader) (1801) 122, t. 1, F. 2. – *Davallia* sect *Wibelia* Kato, J. Fac. Sci. Univ. Tokyo. Sect. 3 (13) (1985) 566. – Type species : *Wibelia elata* Berhn.

Scyphularia Fée, Mém. Foug. 5. Gen. Filic. (1852) 324, t. 26B, f. 1. - Type species: *Scyphularia pentaphylla* (Blume) Fée.

Roots restricted to the ventral side of lateral buds. Scales of rhizome peltate or with cordate base and overlapping lobes (pseudopeltate), variously shaped: distinctly acicular, flat and nearly acicular, narrowed evenly towards the apex, narrowed abruptly from a broad base, or broad, ovate to oblong-subdeltoid with round to acute apex. Lamina deltoid and broadest towards the base or rarely elongate, glabrous or rarely bearing multicellular hairs. Vein endings on sterile segments reaching the margin or not. False veins present or not. Rachis winged and therefore seemingly grooved adaxially. Indusia semi circular or pouch shaped. Sori at the forking point of veins or at the bending point of a vein.

Key to species

1.a	Rhizome small in diameter (<5mm), white waxy surface	2
1b	Rhizome large >5mm, no white waxy layer	8
2a.	Lamina divided into very fine linear segments	**4. *D. parvula***
2b	Lamina not divided into very fine linear segments.....	3
3a	Fronds bipinnate, indusium attached at base and side	**2. *D. corniculata***
3b.	Fronds simple, pectinate, indusium attached at base only	4
4a.	Sterile fronds simple and entire, fertile deeply lobed	**3. *D. heterophylla***
4b.	Sterile and fertile fronds similarly lobed	5
5a.	Fronds simple and entire	**1. *D. angustata***
5b.	All fronds deeply lobed	6
6a.	Fronds lobed almost to the midrib, entire except the basal pinnae lobed at basiscopic side.....	**5. *D. pectinata***
6b.	Fronds lobed almost to the midrib, main lobes with many secondary lobes	7
7a.	Fronds tri-lobed, sterile and fertile fronds slightly dimorphic.....	**6. *D. repens***
7b.	Fronds bipinnate to tripinnate, sterile and fertile fronds strongly dimorphic	**7. *D. vestita***
8a.	Rhizome flattened, fronds trifoliate	**14. *D. triphylla***
8b.	Rhizome rounded, fronds bipinnate to tripinnate	9
9a.	False veins present between true veins	10
9b.	No false veins between true veins	12
10a.	Rhizome large, more than 1 cm diameter, lamina texture thick or coriaceous	**8. *D. denticulata***
10b	Rhizome small 5-8 mm, lamina texture thin or herbaceous	11

11a.	Rhizome scales red brown, with short lateral hairs	**13. *D. trichomanoides* var. *trichomanoides***
11b	Rhizome scales black, long pale lateral hairs	**12. *D. trichomanoides* var. *lorrainii***
12a.	Fronds not dimorphic	**11. *D. solida***
12b	Fronds strongly or slightly dimorphic	13
13a.	Fronds strongly dimorphic, one sorus on each lobe, indusium wider than long	**9. *D. dimorpha***
13b	Fronds slightly dimorphic, several sori on each lobe, indusium as wide as long	**10. *D. divaricata***

1. Section Humata

Davallia sect. *Wibelia* (Bernh.) Kato, J. Fac. Sci. Uni. Tokyo 3 (13) (1985) 566. *Humata* Cav. Descr. Plant. 272, 1802, sensu stricto. Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 364. –Type: *Humata ophioglossa* Cav.

This section is diverse in size and form of leaves from simple and linear to large, and finely dissected (*D. corniculata*), indusia attached by base only or by base and slightly on side, rhizome with white waxy layer.

1. *Davallia angustata* Wall. ex Hook. & Grev.

Davallia angustata Wall. ex Hook. & Grev., Ic. Fil. T. 231.1831; Noot., Fl. Mal. Ser. 2. 3 (1998) 247. - *Humata angustata* (Wall.) J. Sm., Journ. Bot. 3:416. 1841. Bedd., Handb. 47; F.B.I. t.237; Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 367.- *Pachypleuria angustata* C. Presl, Epim. Bot. (1851) 98. –Type: Wallich 242 (K holo), Roxburgh, Prince of Wales I.
Humata angustata var. *hastata* C. Chr., Gard. Bull. Str. Settlem. 4 (1929) 398. – Type: Henderson 18256 (BM, SING), Peninsular Malaysia, Pahang, P. Tioman, G. Kajang.

Rhizome 1-2.5 mm thick with white waxy layer. Scales red-brown to nearly black, without pale border, narrowed evenly towards the apex, toothed, peltate, not bearing multiseptate hairs. Stipes pale to dark brown, adaxially grooved, 1-7 cm long, glabrous or with few scales. Fronds simple, entire to pinnatilobed, linear, glabrous, 5-24 cm long by 6-20 cm broad, not or slightly dimorphic.

Margins distinctly crenulate to dentate at least towards apex. False veins absent. Sori separate at the forking point of veins. Indusium attached at the broad base and hardly or not at the sides, semicircular.

Distributions: Johore, Kedah, Kelantan, Melaka, Negeri Sembilan, Pahang, Penang, Perak, Selangor and Terengganu

Ecology: Epiphytic, often low on tree or epilithic.

Specimens examined:

Johore, Gunung Panti, Bidin et al, AB 1897 [UKMB], RE Holttum 17691 [SING], Maxwell 81-171 [SING]; RE Holttum 17691 [SING]; Kota Tinggi Water Falls, Bidin et al, AB 1923 [UKMB], BS Parris 10987 [K]; Sungai. Berlindung, RE Holttum 10873 [SING]; Tebrau HN Ridley s.n [SING]; Gununug Ledang, HN Ridley 3336 [SING]; Gunung Bercha, RE Holttum 10840 [SING]; Teluk Pulai, HN Ridley s.n. [SING]

Kedah, Gunung Jerai, Damanhuri and H Maideen HM6023 [UKMB]; HN Ridley 5179 [SING].

Kelantan, S. Tekal, Gua Ninik, MR Henderson 19727 [SING].

Melaka, Gunung Ledang, HN Ridley 3336 [SING].

Negeri Sembilan, Gunung Angsi, G Ghouse 4822 [K]; Md Nur 11616 [SING]; Gunung Telapak Burok, Piggott AG1951 [K]; Ulu Pedas Md.Nur 11730 [SING].

Pahang, Fraser Hills, H Maideen and R Jaman HM6006, HM6008 [UKMB]; T Shimizu M13864 [SING]; Kuala Teku, E.Seimund 372 [SING]; Sungai Sat, MR Henderson 21996 [SING]; Tioman Island, Gunung Kajang, R Jaman and Z Mohd, RJ2963 [UKMB]; MR Henderson 18943 [SING]; Lanchang, R Jaman and Z. Mohd, RJ 1811 [UKMB]; Fraser Hills, Willis R Littke, 01867 [UKMB].

Penang, RE Holttum 8983 [SING]; Dr Mateir s.n [SING]; Penang Hill, BM Allen 1547 [SING]; Penang Hill to Tiger Hill, T. Shimizu et al. M 13146 [SING]; Water fall, Md Nur s.n [SING].

Perak, MR Henderson s.n [SING]; Buyung Malaka, HN Ridley s.n [SING].

Selangor, Batu 15 Pahang track, HN Ridley 8647 [SING]; Frasers Hill, T. Shimizu et al. M 13864 [SING]; Genting Simpah, HL Hume 9667 [SING].

Terengganu, Gunung Padang, L. Moysey 33373 [SING].

2. *Davallia corniculata* T. Moore

Davallia corniculata T. Moore, Index Fil. (1861) 292; Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 359; Noot., Fl. Mal. Ser. 2. 3 (1998) 249.–Type: Lobb 220 (BM), Java.

Rhizome 3-4 mm thick with white waxy layer. Scales red-brown, narrowed evenly towards the apex, not bearing multiseptate hairs, with marginal setae at least in distal part, peltate. Stipe dark brown, adaxially grooved, 9-30 cm long,

glabrous or with few scales. Fronds compound, bipinnate or tripinnate towards base and in the middle part, deltoid and broadest towards base, glabrous, 16-50 cm long by 9-25 cm broad, not or slightly dimorphic. Lamina extending into tooth at both side. Veins in sterile frond reaching the margin. False veins present. Sori separate, borne several on a segment at the forking point of veins. Indusium attached at the base and only part of the sides, or also attached along the sides, pouch-shaped, more or less triangular to rhomboid or oblong, about as wide as long, ca. 0.5 mm long and broad, upper margin not elongated, truncate or slightly rounded.

Distributions: Negeri Sembilan, Pahang, Perak, Selangor and Terengganu

Ecology: Epiphytic or epilithic, sometimes in rather dry places. Altitude 300-1800 m.

Specimens examined

Negeri Sembilan, Gununung Telapak Burok, AG Piggott 2641 [SING].

Pahang, Cameron Highland, Robinson's fall, H Maideen and R Jaman HM6051 [UKMB]; MR Henderson 23253 [SING]; RE Holttum 31296 [SING]; Track to Gunung Brinchang, H Maideen and R Jaman HM6054 [UKMB]; Fraser Hills, RE Holttum 21531 [SING].

Perak, Bukit Larut, H Maideen and R Jaman HM6032 [UKMB]; Gunung Batu Putih, Dr Kings collector 8037 [SING]; Maxwell Hill (Bukit Larut), J Sinclair 5995 [SING]; Birch Hill, IH Burkill 12995 [SING];J Sinclair & Kiah 38605 [SING].

Selangor, Pulau Angsa, M Kasim & A Rahim s.n [UKMB].

Terengganu, Jambu Bongkok, M Kasim & A Rahim s.n [UKMB].

3. *Davallia heterophylla* Sm.

Davallia heterophylla Sm., Mém. Acad. Sci. Turin 5 (1793) 415; Noot., Fl. Mal. Ser. 2. 3 (1998) 254. -*Humata heterophylla* (Sm.) Desv., Prod. Fam. Foug. (1827) 323; Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 366.-Lectotype: Charles Miller 17778 (BM).
Davallia longicauda H. Christ, Bot. Jahrb. Syst. 23 (1896) 339. – Type: Wallich 241 (K), Malaya, Penang.

Rhizome 1.8-2.4 mm thick with white waxy layer. Scales red-brown, narrowed evenly towards the apex or flat and nearly acicular and narrowed abruptly from a broad base, not bearing multiseptate hairs, with marginal setae at least in distal part, peltate. Stipes pale, adaxially grooved, 0.5-7 cm long, glabrous or with few scales. Fronds simple, entire to pinnatilobed, bearing multicellular hairs or glabrous, strongly dimorphic. Sterile fronds narrowly ovate, 5-20 cm long by 20-45 mm broad, margin flat or nearly so, not distinctly crenulate even towards apex. Fertile fronds linear or rarely pinnatifid, 4-16 cm long by 5-25 mm broad. False veins not present. Sori separate at the forking point of veins. Indusium attached at the broad base and hardly or not at the sides, semicircular,

wider than long, upper margin not elongated, truncate or slightly rounded, extending to lamina margin.

Distributions: Johore, Kedah, Kelantan, Pahang, Perak and Selangor

Ecology: Epiphytic or epilithic, sometimes in swamp forest.

Specimens examined

Johore, Batu Pahat, Bukit Patani, Ridley s.n [SING]; Gunung Belumut, RE Holttum 10669 [SING]; Manai-Sedili road, Chew,WL CWL 221 [SING]; Sedili, EJH Corner 30862 [SING]; Sedili, Kiah 31988 [SING]; Sungai Berlindung, RE Holttum 10881 [SING].

Kedah, Gunung Jerai, H Maideen and R Jaman HM6024 [UKMB]

Kelantan, Gua Musang, Anon 230 [UKMB]; R Jaman ans S. Miran, RJ 1990 [UKMB], MR Henderson 2264 [SING]; Gua Ninik, MR Henderson 19527 [K]; Temenggong, Md Haniff & Md Nur 10171 [SING]..

Pahang, Cameron Highland, Tanah Rata HN Ridley s.n [SING]; Pekan, HN Ridley 2160 [SING]; Tioman Island, Gunung Kajang, Sidek Kiah & Maulod Elin SK 539 [UKMB].

Perak, Dr Kings collector 1821 [SING].

Selangor, Bukit Batu Berdinding, Md Nur 34343 [SING]; Klang Gates, Turnau 852 [KLU].

4. *Davallia parvula* Wall. ex Hook. & Grev.

Davallia parvula Wall. ex Hook. & Grev., Icon. Filic. (1829) t. 138; Noot., Fl. Mal. Ser. 2. 3 (1998) 257. – *Humata parvula* (Hook. & Grev.) Mett., Fil. Hort. Bot. Lips. (1856) 102, t. 27,f. 7, 8; Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 369; - *Leucostegia parvula* (Hook. & Grev.) Bedd., Handb. Ferns Brit. India (1883) 54. – Type: Wallich 247 (K,BM, L) Singapore.

Rhizome 0.5-1.2 mm thick with white waxy layer. Scales red-brown, narrowed evenly towards the apex, not bearing multiseptate hairs, with marginal setae at least in distal part, peltate. Stipes dark brown, adaxially grooved, glabrous. Fronds compound, entirely divided into fine linear segments, deltoid and broadest towards base, glabrous, slightly dimorphic. Veins in sterile ultimate lobes frequently simple, reaching the margin. False veins not present. Sori separate, frequently single on a segment at the forking point of veins. Indusium attached at the broad base and hardly or not at the sides, semicircular to rhomboid, about as wide as long, upper margin not elongated, truncate or slightly rounded, extending to lamina margin or not. Lamina generally extending into a tooth at both sides of a sorus.

Distribution: Collected in Johore and Singapore.

Ecology: Epiphytic or epilithic in mangrove

Specimens examined
Singapore, Anon s.n [SING]; Kranji, HN Ridley 87[SING].

5. *Davallia pectinata* Sm.

Davallia pectinata Sm., Mém. Acad. Sci. Turin 5 (1793) 415; Noot., Fl. Mal. Ser. 2. 3 (1998) 258; - *Humata pectinata* (Sm.) Desv., Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 369.– Type: D. Hurlock 1786 (n.v), 'India Orientalis'.

Rhizome 1.4-2.6 mm thick with white waxy layer. Scales red-brown, narrowed evenly towards the apex, bearing multiseptate hairs at least when young, peltate. Stipes pale or dark brown, adaxially grooved, 5-18 cm long, glabrous or with few scales. Fronds pectinate or pinnatifid, narrowly ovate, elongate, often narrowing towards base, bearing multicellular hairs or glabrous, 4-21 cm long by 2.5-8 cm broad, not or slightly dimorphous. False veins not present. Sori separate at the forking point of veins or at the bending point of a vein. Indusium attached at the broad base and hardly or not at the sides, semicircular, wider than long or about as wide as long. Upper margin not elongated, truncate or slightly rounded, extending to the lamina or not. .

Distributions: Johore, Kelantan, Langkawi Islands, Melaka, Pahang and Terengganu

Ecology: Epiphytic, epilithic, or sometimes terrestrial, on sand or limestone.

Specimens examined
Johore, Teluk Ayam Pulai, HN Ridley 13277 [SING].
Kedah, Gunung Jerai, Damanhuri and H Maideen HM6022 [UKMB]; H Maideen and R Jaman HM6026 [UKMB]; Langkawi Island, C Curtis s.n [SING]; HC Robinson 6401 [K]; R Jaman and A Bidin, PL 145 [UKMB]
Kelantan, Anon 672 [UKMB]; Gua Panjang, MR Henderson 19538 [SING].
Melaka, HN Ridley s.n [SING]; J. Sinclair 40582 [SING].
Pahang, Pekan, HN Ridley s.n [SING]; Rompin river, JHN Evans s.n [K].
Terengganu, Pulau Kapas, RE Holttum & Md Nur 15208 [SING].

6. *Davallia repens* (L.) Kuhn

Davallia repens (L.) Kuhn. Ann. Mus. Bot. Lugd. Bat. 4 (1869)286; Noot., Fl. Mal. Ser. 2. 3 (1998) 259.- *Humata repens* (L. f.) Diels, Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 371.

Rhizome 1.0-2.0 mm thick with white waxy layer. Scales brown or red-brown, narrowed evenly towards the apex, bearing multiseptate hairs at least when young or with marginal setae at least in distal part, peltate. Stipes adaxially grooved, 0.1-18 cm long, glabrous or with few scales. Fronds compound, simple (3-lobes) or pinnate towards base, ovate, deltoid and broadest towards

base, glabrous. Strongly dimorphic or not or slightly dimorphic. In dimorphic plants, fertile leaves pinnate with strongly dissected pinnae, bipinnate, or tripinnate towards base and in the middle part. Veins in sterile lobes simple, forked, or pinnate, reaching the margin. False veins not present. Sori separate, borne several on a segment, or in much divided leaves frequently single on a segment, at the forking point of veins. Indusium attached at the broad base and not at the sides, semicircular or more or less triangular to rhomboid, wider than long, upper margin not elongated, truncate or slightly rounded, extending to lamina margin or not. Lamina generally extending into a tooth at both sides.

Distributions: Johore, Kedah, Kelantan, Pahang, Penang, Perak and Selangor.

Ecology: Epiphytic and epilithic on various kinds of rocks.

Specimens examined

Johore, Gunung Belumut, RE Holttum 10668 [SING]; Gunung Ledang, Sungai Ayer Panas, Parris 10996 [K]; Gunung Panti, J Sinclair 4764 [UKMB]; Md Nur 20029 [SING]; Mt. Ophir, Piggott 1747 [K]; Tanjung Selantai, RE Holttum 24968 [SING].

Kedah, Gunung Jerai, H Maideen and R Jaman HM6019, HM6021, HM6025 [UKMB]; Anon 8 [UKMB]; HN Ridley 5179 [SING]; VL Gurung 43 [SING]; Davis 94417 [SING]; Pulau Langkawi, G. Machinchang, Burtt & Woods s.n [SING]; G. Raya, C Curtis s.n [SING].

Kelantan, Gua Musang, MR Henderson 22653 [SING]; Kuala Condong, Md Haniff & Md Nur 10164 [K].

Pahang, Cameron Highland, Boh plantations, Md.Nur 32850 [SING]; Tanah Rata, T. Shimizu et al. M 13555 [SING]; Frasers Hill, H Maideen and R Jaman HM6002, HM6007 [UKMB]; BM Allens 1426 [UKMB]; GH Addison 37206 [SING]; IH Burkil & RE Holttum 8463 [SING]; MR Henderson11281 [SING]; RE Holttum s.n [SING]; Genting Highland, Gunung Buah track, Parris 10546 [K]; Gunung Tahan, HN Ridley 16004 [SING]; RE Holttum s.n [SING]; Wray & Robinson 5437 [SING]; Kuala Teku, E.Seimund 385 [SING]; Tioman Island, G. Rokam, MR Henderson 18795 [SING]; Taman Negara, Bkt Indah, Parris & Edwards 10478 [K].

Penang, RE Holttum s.n [SING]; Penang Hill, Hardial & Samsuri 191 [SING]; T Shimizu et al. M 13045 [SING]; VL Grorng 82 [SING]; RE Holttum 19775 [SING].

Perak, Bukit Larut, H Maideen and R Jaman HM6033, HM6035 [UKMB]; Wray s.n [SING]; Bukit Larut, FEW Venning MA 95 [K]; J Sinclair & Kiah 38625 [SING]; J Sinclair 6015 [SING]; J. Sinclair & Kiah 38625 [SING].

Selangor, Bukit Batu Berdinding, Md.Nur 34349 [SING]; Fraser Hill, Burkill & Holttum 8643 [K]; Kajang, Symington 24157 [KEP].

7. *Davallia vestita* (Blume) T. Moore

Davallia vestita Blume, Enum. Pl. Jav. (1828) 233. - *Humata vestita* (Blume) T. Moore, Index Fil. (1857) 92; Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 370; - Type Blume s.n. 908.275-969 (L), Java.

Rhizome slender with 1.0-2.0mm thick with white waxy layer. Scales brown, peltate, toothed or bearing short hairs. Stipes up to 15 cm long, bearing scales, upper surface grooved to base. Fronds dimorphic, broadly to deltoid or bipinnate to tripinnate at the base. Sterile pinnae with coriaceous blunt elliptical rather narrow slightly toothed segments; fertile pinnae with lamina much reduced. Sori small. Indusium attached at base, reniform or circular.

Distribution: Only collected in Pahang

Ecology: Terrestrial in shaded area.

Specimens examined

Pahang, Gunung Tahan, L Wray & HC Robinson 5472 [SING]; Cameron Highland, Gunung Brinchang, H Maideen and R Jaman HM6039 [UKMB]; MR Henderson 17989 [SING]; RE Holttum 31357 & 23287 [SING].

2. Section Davallia

Davallia sect. *Davallia*, Kato, J. Fac. Sci. Uni. Tokyo 3 (13) (1985) 566; - *Davallia*, Holttum, Rev. Fl. Malaya, ed. 2, 2 (1968) 354.

This section is characterized by a combination of strongly divided leaves, large rhizome, scales not acicular to nearly acicular, indusia attached at base and along sides, pouch-shaped.

8. *Davallia denticulata* (Burm. f.) Kuhn

Davallia denticulata (Burm. f.) Mett. ex Kuhn, Fil. Decken. (1867) 27; Holttum, Rev. Fl. Malaya, ed. 2, 2 (1968) 359; - *Davallia denticulata* var *denticulata* Noot., Fl. Mal. 2, 3. (1998) 250; - *Humata elegans* (Sw.) Desv., Prod. Fam. Foug. (1827) 324.

Rhizome stout, 0.5-1.3 cm thick. Scales light brown, narrowed gradually from peltate base, toothed. Stipe pale, adaxial grooved, glabrous. Fronds compound, bipinnate to quadripinnate, deltoid and broadest towards the base, slightly dimorphic. Pinnae deltoid. Veins in sterile frond pinnate, reaching the margin. False veins present. Sori several on a segment, at the forking point of veins. Indusium pouch-shaped, oblong, upper margin not elongated, truncate or rounded. Fronds extending into a tooth at both side of sorus.

Distributions: Johore, Kedah, Kelantan, Melaka, Negeri Sembilan, Pahang, Penang, Perak , Selangor and Terengganu .

Ecology: Epiphytes on trees, mostly on palm oil trees. Alt. 0-1000 m

Specimens examined

Johore, Gunung Ledang, AG Piggott AG1437 [K].

Kedah, Gunung Jerai, H Maideen and R Jaman HM6028 [UKMB]; HN Ridley 5159 [SING]; Pulau Langkawi, G. Raya, R Jaman & A Zainuddin PL 73, PL232, PL197 [UKMB]; MR Henderson 2908 [SING].

Kelantan, Gua Musang, Anon 627, R. Jaman RJ1938, RJ1947 [UKMB].

Melaka, Pulau Jarak, Wyatt Smith 71044 [KEP]; Sungai Udang HN Ridley 1644 [SING].

Negeri Sembilan, Ulu Sepri, H Maideen and R Jaman HM6016a [UKMB]; Anon s.n [UKMB]; Tanjung Tuan, A.Bidin s.n [UKMB]

Pahang, Frasers Hill, WR Littke W143 [UKMB]; Taman Negara, Ulu Tembeling, A Bidin & R Jaman AB 1694 [UKMB]; Cameron Highlands A. Samad s.n [UKMB]; Rompin, IHN Evans958, 959 [SING]; Kuala Tahan, E Seimund 64 [SING]; Tioman Island, MR Henderson 18418 [SING].

Penang, Penang Hill, T. Shimizu et al. M 13128 [SING]; VL Gurung 68b [SING]; M Nur s.n. 57902 [SING].

Perak, Lalang Island, E.Seimund s.n [SING]; Pangkor Island, L. Sahid and S. Miran LS 83, LS 111 [UKMB]; Batu Senai, MR Henderson 19432 [SING]

Selangor, Universiti Kebangsaan Malaysia campus, H Maideen and R Jaman HM6016b [UKMB]; Genting Simpah, HK Hume 9182 [UKMB]; Angsa Island, HM Burkil & Md Shah HMB 931 [SING]; Templer park, WR Littke W192 [UKMB], Ismail Sahid, IS 149, 190, 194 [UKMB]; Angsa Island, Zulkifli et al ZM 163 [UKMB]; Jarak Island, J Wyatt-Smith 71044 [SING].

Terengganu, Dungun, A. T Othman AT1 [UKMB].

9. ***Davallia dimorpha*** **Holttum**

Davallia dimorpha Holttum, Gard. Bull. Str. Sett. 9 (1937) 122; Rev. Fl. Malaya, ed. 2, 2 (1968) 362.; - *Araiostegia dimorpha* (Holttum) Kato, Acta Phytotax. Geobot. 26 (1975) 158; - Type: Holttum *SF* 31289 (SING), Peninsular Malaysia, Pahang, Cameron Highlands.- *Davallia divaricata* var. *dimorpha* (Holttum) Noot., Noot., Fl. Mal. 2. 3 (1998) 253.

Rhizome 8-15 mm thick. Scales basifixed with cordate base, thin, brown without pale border. Stipe pale. Adaxially grooved, up to 30-50 cm long. Fronds bipinnate to tripinnate, strongly dimorphic. Sterile frond with deltoid pinnules. Fertile frond much more dissected. Sori broader than long, single on a segment, at the forking point of veins. Indusium crescent-shaped.

Distribution: Only recorded in Pahang.

Ecology: Epiphytes in shaded areas. Alt. 4000-5000 m

Specimens examined

Pahang, Cameron Highland, Gunung Brinchang, H Maideen and R Jaman HM6045 [UKMB]; RE Holttum s.n [UKMB]; Batten Pool s.n [SING].

10. ***Davallia divaricata*** **Blume**

Davallia divaricata Blume, Enum. Pl. Javae (1828) 737; Rev. Fl. Malaya, ed. 2, 2 (1968) 362;
- *Davallia divaricata* var. *divaricata*, Noot., Fl. Mal. 2. 3 (1998) 252.
- *Davallia polyantha* Hook., Sp. Fil. (1845) 168, t. 59A;- Type: Lobb s.n. (K) Singapore.

Rhizome about 2-2.5 cm thick. Scales basifixed with cordate base, thin, chestnut brown, lanceolate, toothed and hairtipped. Stipe 35-40 cm long, grooved above. Frond deltoid, 30-50 cm wide, bipinnate to quadripinnate, pinna and pinnules acuminate, glabrous. Vein forking, no false vein. Sori at the forking point of vein. Indusium pouch-shaped, oblong. Upper margin not elongated, truncate or rounded. Lamina generally extending into tooth at both side of sorus.

Distributions: Langkawi Islands, Negeri Sembilan, Pahang, Perak and Tioman Island

Ecology: Epiphytes in dense forest and in dry places.

Specimens examined

Kedah, Pulau Langkawi, G. Raya, Md Haniff 7125 [SING]; R. Jaman & A Zainuddin PL 54 [UKMB]; Pulau Telor Zulkifli et al. ZM 195 [UKMB].

Negeri Sembilan, Gunung Telapak Burok, AG piggottt 1830 [SING].

Pahang, Cameron Highland, MdNur 32931 [SING]; RE Holttum s.n. [SING]; Fraser Hill, H Maideen and R Jaman HM 6000, HM 6005, HM6012, HM6013, HM6014, HM6015 [UKMB]; RE Holttum 8864 [SING]; Sungai Jeriau, BC Stone 8655 [KLU]; IH Burkill 8846 [SING];Lubok Tamang, MR Henderson 10946 [UKMB]; Tioman Island, Gunung Kajang, R Jaman & Zulkifli M RJ 2865 [UKMB]; Side of Gap, GH Addison 37190 [SING]; Tras valley, RE Holttum 21611 [SING].

Perak, Bukit Larut, H Maideen and R Jaman HM6031 [UKMB]; Larut, Dr Kings collector 2200, 2201 [SING]; J Sinclair 6039 [SING]; J. Sinclair & Kiah 38649 [SING]; Pulau Agas, Lagani & Sani LS 128 [UKMB]; Taiping Hill, Md. Haniff & Md. Nur 2378 [SING].

3. Section Scyphularia

Davallia sect. *Davallia* Kato, J. Fac. Sci. Uni. Tokyo 3 (13) (1985) 566; - *Davallia*, sect. *Scyphularia* (Fée), Noot. Fl. Mal. 2.3. (1998) 266.

This section is characterized by acicular scales, coriaceous leaves and pouch-shaped indusia.

11. *Davallia solida* (G. Forst.) Sw.

Davallia solida (G. Forst.) Sw. J. Bot. (Schrader) (1801) 87; Rev. Fl. Malaya, ed. 2, 2 (1968) 360; Noot. Fl. Mal. 2.3. (1998) 263; .- Type: Wallich 246 (K) Malaya, Penang.
- *Davallia solida* (G. Forst.) Sw. var. *ornata* (C. Presl) Mett. ex Kuhn, Ann. Mus. Bot. Lugd. Bat. 4 (1869) 286; - Type : Forster 308 (BM holo;P), Pacific Islands

Rhizome 6-12 mm thick. Scales red brown, peltate, broad base evenly narrowed towards the apex, bearing multiseptate hairs. Stipes 10-25 cm long, adaxially grooved. Fronds compound, bipinnate to tripinnate., deltoid, glabrous but sometimes with hairs on base of petiole. Fertile frond more deeply lobed. Vein distinct, no false veins. Sori borne several on a segment at the forking point of the veins. Indusium pouch-shaped, oblong, upper margin not elongated, truncate or slightly runded, extending to lamina margin or not. Lamina not extending into teeth beyond a sorus.

Distributions: Johore, Kedah, Kelantan, Pahang, Penang, Perak, Selangor and Terengganu.

Ecology: Epiphytes or terrestrial in open or shaded places.

Specimens examined

Johore, Gunung Belumut, RE Holttum 10880 [SING]; Kluang, RE Holttum 9426 [SING];Aur Island, Dr Kings collector s.n. [SING]; Kota Tinggi, Z Teruya s.n. [SING].

Kedah, Gunung Jerai, H Maideen and R Jaman HM6017 [UKMB]; Damanhuri and H Maideen HM6027 [UKMB]; HC Robinson & Kloss 6045 [SING].

Kelantan, Gua Musang, Razali Jaman RJ 1951 [UKMB].

Pahang Tioman Island, Juara bay EJH Corner s.n [UKMB].

Penang, Penang Hill, H Maideen and R Jaman HM6030 [UKMB].

Perak, Bukit Larut, A Bidin, R Jaman & S Miran AB 2066 [UKMB]; Dr King's coll 7068 [SING]; Pulau Rumbia, E.Seimund s.n [SING]; Lagani & Sani LS 118 [UKMB]; Pulau Samak Lagani & Sani LS 92 [UKMB].

Selangor, Klang Gate, Turnau 853 [SING]; Pulau Angsa, JW Smith 71144 [SING]; HM Burkill 934 [SING].

Terengganu, Jambu Bongkok, Umi Kalsom UK 86 [UKMB]; Kemaman, EJH Corner s.n [UKMB].

12. *Davallia trichomanoides* Blume var. *lorrainii* (Hance) Holttum

Davallia trichomanoides Blume var. l*orrainii* (Hance) Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 361; Noot. Fl. Mal. 2. 3. (1998) 265. - *Davallia lorrainii* Hance, Ann Sci. Nat. Bot. 5 (1866) 254. – Type: Lorrain 1732 (BM), Malaysia, Penang.

Rhizome about 3 mm thick. Scales black, rounded peltate base evenly narrowed towards the apex, margin usually bearing long spreading hairs. Stipe adaxially grooved, glabrous or with few scales. Frond 12- 30 cm long, quadripinnate, deltoid. Sterile fronds unequally bilobed with a vein once or twice forked. Fertile frond with single vein only. False veins present. Sori single on a segment at the forking point of veins. Indusium pouch-shaped, oblong.

Distributions: Kedah, Negeri Sembilan, Pahang, Penang and Selangor.

Ecology: Epiphytes in moist place and open places.

Specimens examined

Kedah, Gunung Jerai, H Maideen and R Jaman HM6018 [UKMB]; VL Gurung 59a [SING]; HN Ridley 5150 [SING]; Langkawi Islands, Gunung Raya, summit, R. Jaman & Hamid RJ 2076 [UKMB]; G. Raya, Md. Haniff & Md. Nur 7102 [SING].

Negeri Sembilan, Gunung Telapak Burok, AG Piggott 1828 [SING].

Pahang, Cameron Highland, Tanah Rata, BM Allen 2914 [SING]; R Jaman and H Salleh, RJ 2076 [UKMB]; Fraser Hills, Jeriau Water Falls, H Maideen and R Jaman, HM6003 [UKMB]; Pekan, HN Ridley s.n. [SING]

Penang, Penang Hill, H Maideen and R Jaman HM6029 [UKMB]; plant house no. 9, Md. Nur s.n [SING]; Unknown s.n. [SING].

Selangor, Fraser Hill, T Shimizu et al. M13851 [KYO].

13. *Davallia trichomanoides* Blume var. *trichomanoides*

Davallia trichomanoides Blume, Enum. Pl. Java (1828) 238; Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 361; -*Davallia trichomanoides* Blume var. *trichomanoides,* Noot. Fl. Mal. 2. 3. (1998) 264;

Rhizome about 3 mm thick. Scales chestnut brown, rounded peltate base evenly narrowed towards the apex, margin usually bearing very short spreading hairs. Stipes adaxially grooved, glabrous or with few scales. Frond 12- 30 cm long, quadripinnate, deltoid. Sterile frond unequally bilobed with a vein once or twice forked. Fertile fronds with single vein only. False veins present. Sori single on a segment at the forking point of veins. Indusium pouch-shaped and oblong. Frond usually extending into a tooth at both side of sorus.

Distribution: Only collected in Pahang and Perak.

Ecology: Epiphytes in moist and open places.

Specimens examined

Pahang, Cameron Highland, Tanah Rata, T Shimizu et al. M13245 [KYO]; G.Brinchang, H Maideen and R Jaman HM6038, HM6044, HM6047 [UKMB]; T Shimizu et al. M13405 [KYO]; Fraser Hills, Jeriau Water Falls, H Maideen and R Jaman, HM6009 [UKMB].

Perak, Taiping, Gunung Hijau, H Maideen and R Jaman HM6041 [UKMB].

14. ***Davallia triphylla*** **Hook.**

Davallia triphylla Hook., Sp. Fil. (1845) 162, t. 46a; Holttum, Rev. Fl. Malaya, ed 2, 2 (1968) 361; Noot. Fl. Mal. 2. 3. (1998) 267- *Scyphularia triphylla* Fée, Gen Fil. (1850) 324;.- Type: Cuming 366 [K], Singapore

Rhizome flattened, about 6 mm wide. Scales nearly black, distinctly acicular, bearing multiseptate hairs at least when young, peltate. Stipes dark brown, adaxially grooved, 2-8 cm long, glabrous. Fronds simple or trifoliate, glabrous, slightly dimorphic. Margin distinctly crenulate to dentate at least towards apex. Pinnae narrowly ovate. Fertile leaves entire or nearly so, sometimes with some basal lobes, or pinnatifid. Veins in sterile leaflets parallel, once or twice branched from the base, reaching the margin. False veins not present. Sori separate at the forking point of veins. Indusium attached along the sides, pouch-shaped, oblong, longer than wide, upper margin not elongated, truncate or slightly rounded, extending to lamina margin or not or protruding beyond lamina margin.

Distributions: Johore and Terengganu

Ecology: Epiphytes on dry places

Specimens examined

Johore, Gunung Pulai, Sungai Air Hitam Besar, H Maideen and R Jaman HM6046 [UKMB]; Zulkifli & Rusea ZM 104 [UKMB]; Gunung Pulai, J Sinclair 39513 [SING].

Terengganu, Ulu Setiu, GP Lewis 79 [UKMB].

List of species used in this study with RBGE accession numbers

Species	Locality	Coll. No.	Herbarium	RBGE accession no.
Araiostegia hymenophylloides	Malaysia, Perak, Bukit Larut track to post off	HM6035	UKMB, E	20051615
D. angustata	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6006	UKMB	
D. angustata	Malaysia, Kedah, Gunung Jerai, summit	HM6020	UKMB, E	20051632/20051633
D. angustata	Malaysia, Kedah, Gunung Jerai	HM6023	UKMB	
D. corniculata	Malaysia, Perak, Bukit Larut near post off	HM6032	UKMB	20051629
D. corniculata	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	HM6051	UKMB, E	20051614b
D. denticulata	Malaysia, Negeri Sembilan, Pedas, Ulu Sepri, palm oil estate	HM6016a	UKMB	
D. denticulata	Malaysia, Kedah, Gunung Jerai, summit	HM6028	UKMB	
D. denticulata	Selangor, Bangi, University Campus	HM6016b	UKMB	
D. dimorpha	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	HM6045	UKMB	
D. divaricata	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6005	UKMB,E	20051627
D. divaricata	Malaysia, Selangor, Fraser Hills, near Gap (chinese temple)	HM6013	UKMB	
D. divaricata	Malaysia, Perak, Bukit Larut near post off	HM6031	UKMB, E	20051631
D. divaricata	Malaysia, Perak, Bukit Larut, near post off.	HM6037	UKMB, E	20051642
D. divaricata	Malaysia, Perak, Bukit Larut, near post off.	HM6042	UKMB	
D. divaricata	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6012	UKMB	
D. divaricata	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6014	UKMB	
D. divaricata	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6015	UKMB	
D. divaricata	Malaysia, Selangor, Fraser Hills, jalan Istana	HM6000	UKMB	

List of species used in this study with RBGE accession numbers (cont.)

D. divaricata	Malaysia, Perak, Bukit Larut near post off	HM6034	UKMB	20051638/20051639
D. heterophylla	Malaysia, Kedah, Gunung Jerai, summit	HM6024	UKMB	
D. pectinata	Malaysia, Kedah, Gunung Jerai, Tangga Kenari	HM6022	UKMB	20051624/20051635
D. pectinata	Malaysia, Kedah, Gunung Jerai, Tangga Kenari	HM6026	UKMB, E	20051635
D. repens	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6002	UKMB	
D. repens	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6007	UKMB, E	20051634
D. repens	Malaysia, Kedah, Gunung Jerai, summit	HM6019	UKMB	20051646
D. repens	Malaysia, Kedah, Gunung Jerai, Tangga Kenari	HM6025	UKMB	20051634
D. repens	Malaysia, Perak, Bukit Larut near post off	HM6033	UKMB, E	20051645
D. solida	Malaysia, Kedah, Gunung Jerai, summit	HM6017	UKMB	
D. solida	Malaysia, Penang, Penang Hills	HM6030	UKMB, E	20051641
D. solida	Malaysia, Kedah, Gunung Jerai, summit	HM6027	UKMB	
D. trichomanoides var. *lorrainii*	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6053	UKMB	
D. trichomanoides var. *lorrainii*	Malaysia, Kedah, Gunung Jerai, summit	HM6018	UKMB, E	20051640
D. trichomanoides var. *lorrainii*	Malaysia, Penang, Penang Hills	HM6029	UKMB	20051644
D. trichomanoides var. *lorrainiii*	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	HM6003	UKMB	20051621
D. trichomanoides var. trichomanoides	Malaysia, Pahang, Fraser Hills, Valley rd	HM6009	UKMB	
D. trichomanoides var. *trichomanoides*	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	HM6038	UKMB	20051623

List of species used in this study with RBGE accession numbers (cont.)

D. trichomanoides var. *trichomanoides*	Malaysia, Perak, Gunung Hijau, track to summit	HM6041	UKMB	20051613
D. trichomanoides var. *trichomanoides*	Malaysia, Pahang, Cameron Highlands, track Robinson fall	HM6047	UKMB	20051618
D. trichomanoides var. *trichomanoides*	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	HM6054	UKMB	20051614a
D. trichomanoides var. *trichomanoides*	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	HM6044	UKMB, E	20051625
D. trichomanoides var. *trichomanoides*	Malaysia, Perak, Bukit Larut	HM6052	UKMB, E	20051626
D. triphylla	Malaysia, Johore, Gunung Pulai	HM6046	UKMB, E	20051619/20051622
D. vestita	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	HM6039	UKMB, E	20051616
Leucostegia immersa	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	HM6040	UKMB, E	20051617

Molecular Methods

1. The collected fresh plant material in the field was preliminarily cleaned using water and dried with tissue paper before being placed in silica gel filled bags. Samples were then stored in silica gel until required for DNA extraction.

2. **DNA Extraction Protocol using CTAB method (modified from Doyle & Doyle (1990).**

A small amount of plant of silica dried material was placed in a 1.5 ml eppendorf tube with one grinding bead (3mm Reatsch cone ball).

The tissue was ground to a fine powder using Mixer mill (2x 30 sec at 30/sec frequency).

1 ml Preheated CTAB buffer (2 % CTAB, 20 mM EDTA, 100 mM tris-HCl pH 8.0, 1.4 M NaCl and 0.2 % mercaptoethanol at 65^0C) and a pinch of PVPP (PolyVinylPolyPyrolidine) was added to each sample/tube, shook gently before incubated at 65^0C for 30 minutes in heated block.

The tube was removed from heated block and allowed to cool.

500 µl of chloroform isomyl alcohol (24:1) was added to each sample and mixed on an orbital shaker for 15 minutes at minimum speed.

Samples were centrifuged for 10 minutes at 13 000 revolutions per minute (rpm) to separate into two phases.

Supernatant (upper layer) was removed to a clean 1.5 ml eppendorf tube (which was prepared earlier with same label). The chloroform extraction was repeated.

Finally the supernatant was transferred to another clean 1.5 ml eppendorf tube and the DNA precipitated by adding 600µl of cold isopropanol (about 2/3 volume) and mixed gently.

The samples were left overnight at -20°C to allow maximal DNA precipitation.

The tubes were then centrifuged at 13 000 rpm (10 min) to pellet the DNA.

The supernatant was removed and pellet was washed with 1ml wash buffer (70 % ethanol) for at least 30 min at room temperature.

The samples were then centrifuged again for 5 minutes at 13 000 rpm and the supernatant removed.

Pellets were dried, using a vacuum centrifuge for 4 minutes at low dry rate.

Then the pellet was dissolved in 75 µl of TE and mixed well. 5 ul of the DNA from all samples were run on agarose gel to check the quality and concentration.

3. **PCR amplification**

Primers

The following primers were used to amplify all the three regions in this study (Table5.1)

Table 5.1 . Primers used in PCR and sequencing reactions

Region	Primer	Direction	Sequence	References
trnL-F	Fern1	forward	5'-GGC AGC CCC CAR ATT CAG GGR AAC C-3'	Trewick et al., 2002
	trnL-d	reverse	5'-GGG GAT AGA GGG ACT TGA AC-3'	Taberlet et al., 1991
	trnL-e	forward	5'-GGT TCA AGT CCC TCT ATC CC-3'	Taberlet et al., 1991
	trnL-f	reverse	5'-ATT TGA ACT GGT GAC ACG AG-3'	Taberlet et al., 1991
Rps4-Trns	rps4F	forward	5'-ATG TCM CGT TAY CGA GGR CCT CGT-3'	Nadot et al., 1994
	trnas	reverse	5'- TAC CGA GGG TTC GAA TC-3'	Smith & Cranfill, 2002
rbcL	rbcL1F	forward	5'- ATG TCA CCA CAA ACA GAR ACT AAA GC-3'	Gastony & Rollo, 1995
	rbcL135IR	reverse	5'- CTT CAC AAG CAG CAG CTA GTT CAG GAC TCC-3'	Gastony & Rollo, 1995

Reaction conditions
Polymerase Chain Reaction (PCR) was used to amplify the regions.

PCR reactions were performed in 25 or 50 µl per sample using the following recipe:

Reagents	Quantity (µl)
10x Reaction NH4 buffer	2.5
dNTPs (0.2 mM)	2.5
MgCl2 (50 mM)	1.25
forward primer (10mM)	0.75
reverse primer (10mM)	0.75
Biotaq DNA polymerase	0.125
distilled water	10.125-16.625
DNA	0.5-3
Total	25

The PCR program:

Temperature	Time	Number of cycles
94°C	4 min	x1
94°C	45 sec	
55°C	45 sec	x30
72°C	3 min	
72°C	10 min	x1
10°C	forever	

4. **PCR amplification was checked using 1% agarose gels.**
Resultant products were purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, UK).

The purified products were rechecked again on a 1 % agarose gel.

5. **Sequencing**
Sequencing PCRs were performed using the sequencing protocol.
For *trnL-F* region , primer Fern1 (Trewick et al., 2002) and primer f (Taberlet et al., 1991) as external primers and two internal primer d and e (Taberlet et al., 1991) were used.

Sequencing PCR recipe
The sequencing reactions (half reaction) were performed using the purified PCR products and were carried out in 10 µl reactions.

Reagents	Quantity
Distilled sterile water	2–4
DTCS Quickstart mix* (Dye Terminator Cycle Sequencing)	4
Primer (10mM)	1
DNA template	1-3

In some samples, betaine has been added to obtained good sequence
Sequence amplifications were achieved using a Perkin Elmer Thermocycler PTC-200 PCR machine using the following programme:

Temperature	Time	Number of cycles
96°C	20 sec	
50°C	20 sec	35
60°C	4 min	
4°C	forever	

6. **Sequence reaction purification**

The sequence reactions were purified as follows:
All reactions were made up to 20 µl with dH2O and transferred to a clean 0.5 ml microfuge tube.

5 µl of stop solution (stock B) (as below) and 60 µl of 100 % ice cold ethanol were added to each tube.

Stock B (stop solution) (Been prepared fresh each time)	Volume per reaction/tube (µl)
0.5M EDTA	0.4
Sigma water	1.6
3M NaOAc pH 5.2	2
Glycogen	1

The samples were mixed using a vortex mixer and centrifuged for 15 minutes at 4°C at 14 000 rpm.

The supernatant was carefully removed and 200 µl of 70 % ice cold ethanol was added and the samples centrifuged for 5 minutes at 4°C at 14 000 rpm. (repeated twice)

After removing the supernatant, the remaining pellet was vacuum dried on a low drying rate for 4 minutes until no trace of ethanol remained. Each pellet was then resuspended in 35 µl of sample loading solution (SLS) and mixed using a vortex mixer.

Samples were stored at -20°C for analysed using CEQTM 8000 Analyses System DNA Sequencer by staffs at RBGE.

Analysed sequences were edited using CEQTM 8000 Genetic Analysis System Version 7.0 Software and aligned in SequencherTM Version 4.5.

The contig sequences were exported as text files for phylogenetic analysis.

Contig sequences obtained from Sequencher were BLAST searched using NCBI (National Centre of Biotechnology Information) before exported to MacClade version 4.6 (Maddison and Maddison, 2003) for alignment.

List of Davalliaceae and the Genbank accessions numbers used in expended *rbcL* analysis

No	Species	GenBank accessions numbers
1	*Arthropteris_backleri*	AB212686
2	*Araiostegia_faberiana*	AB212688
3	*Araiostegia_hymenophylloides*	AB212689
4	*Araiostegia_perdurans*	AB212691
5	*Araiostegia_pulchra*	AB212692
6	*Araiostegia_yunnanensis*	AB212693
7	*Davallia_aff._mariesii*	AB212706
8	*Davallia_clarkei*	AY096194
9	*Davallia_corniculata*	AB212697
10	*Davallia_denticulata_1*	AB212698
11	*Davallia_denticulata_2*	AB212699
12	*Davallia_divaricata*	AB212700
13	*Davallia_embolostegia*	AB212701
14	*Davallia_epiphylla_1*	AB212702
15	*Davallia_fejeensis*	AB212703
16	*Davallia_formosana*	AB212704
17	*Davallia_griffithiana*	AB212705
18	*Davallia_mari.var._stenolepis*	AB212708
19	*Davallia_mariesii_2*	U05617
20	*Davallia_petelotii*	AB212709
21	*Davallia_plumosa*	AB300576
22	*Davallia_pyxidata*	AB212711
23	*Davallia_solida_1*	AB212712
24	*Davallia_solida_2*	AY096193
25	*Davallia_tasmani*	AB212713
26	*Davallia_trichomanoides*	AB212714
27	*Davallia_tyermanii*	AB212715
28	*Davallodes_borneense*	AB212694
29	*Davallodes_burbidgei*	AB212695
30	*Davallodes_gymnocarpum*	AB212696
31	*Humata_aff._parvula*	AB212719
32	*Humata_aff._polypodioides*	AB212721
33	*Humata_banksii*	AB212716
34	*Humata_melanophlebia*	AB212717
35	*Humata_parvula*	AB212718
36	*Humata_polypodioides*	AB212720
37	*Humata_vestita*	AB212722
38	*Oleandra_wallichii*	AB212689
39	*Scyphularia_pentaphylla*	AB212723
40	*Scyphularia_pycnocarpa*	AB212724
41	*Scyphularia_triphylla*	AB212725