

## CHARACTER ASSESSMENT OF SPECIES GROUP OF MALESIAN ASPLENIACEAE USING MOLECULAR INFERENCE

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Aspleniaceae is one of the biggest families in ferns and is composed of circa 750 taxa. The family is known having world-wide distribution ranging from humid tropical to arid zones (Kramer and Viane 1990). With about 30% of total species in Malesia (Rambe 2002), the region was assumed to be the center of diversity for the family. If a sizeable number of species is endemic to islands (Kramer and Viane 1990), some have reached cosmopolitan distribution. For example, *A. nidus* is spreading from coastal East Africa and Madagascar, over the Indian subcontinent and Malesia, to Australia and islands of the Pacific (Johns 1996). The species occupy various habitats, ranging from the sea level to the top of mountains including the border of volcano craters as illustrated by *A. malayo-alpinum* on Mt. Gede-Pangrango National Park, Indonesia. Many Aspleniaceae species are either epiphyte on basal trunks or occupy the upper canopy area such as *A. nidus*. Few of them are obligated epiphytes (e.g. *A. perakense* and *A. scortechinii*), others are specific to lime stone rock (e.g. *A. adiantoides*), and some have specialized to river banks such as *A. subaquatile* (Kramer and Viane, 1990) and *A. amaroulobulum*.

Members of Aspleniaceae reproduce either sexually and asexually. Asexual reproduction occurs via apogamy or the direct production of young plantlet called gemma (plural gemmae). Apogamy is a formation of new sporophytes without fertilization by the cells of the prothallus. Gemma develops on the frond either near to the rachis, the costa or the vein. The production of gemma is often observed in relation to high moisture and low light intensity. Branching and continuous growth of rhizome is also a natural mechanism of propagation that is commonly used for production in nurseries. In nature, branching rhizomes often form a wide surface coverage commonly observed on many *Hymenasplenium* species. For *Asplenium*, a good example is *A. contiguum* from Gede-Pangrango National Park – Indonesia where a single continuous rhizome was found covering a surface of 5m<sup>2</sup>.

Asplenon was among the ferns mentioned alleviating sleep disorder by Dioscorides in the De Materia Medica (Ruellio 1539) published in the first century (Adanson 1763). In its current form, the name of *Asplenium* is derived from "a", as privative prefix, and "spleen" meaning "sleep" on the account of the supposed property of the fern for curing sleep afflictions (Schneider 1892). It was Linnæi (1738) that proposed this name for ferns with linear sori (singular sorus) encompassing the ones cited

(Figure 1) collected by Pehr Osbeck from Java (Linnæi 1753) close to sea shore area of formerly Sunda Kelapa port. The period following the work of Linnæi from Mid 1700s to the beginning 1900s was the golden era of plant collection. Newly collected fern specimens were proposed as *Asplenium* when linear sorus was the only distinctive character retained. However, new genera were given when linear sori were associated with other morphological characters or the linear sorus was in form of boat shape. Henceforth, based on various morphological characters, there were more than twenty genera proposed within the ferns having linear sorus. These genera were later compiled and proposed as Aspleniaceae by Frank (1877).

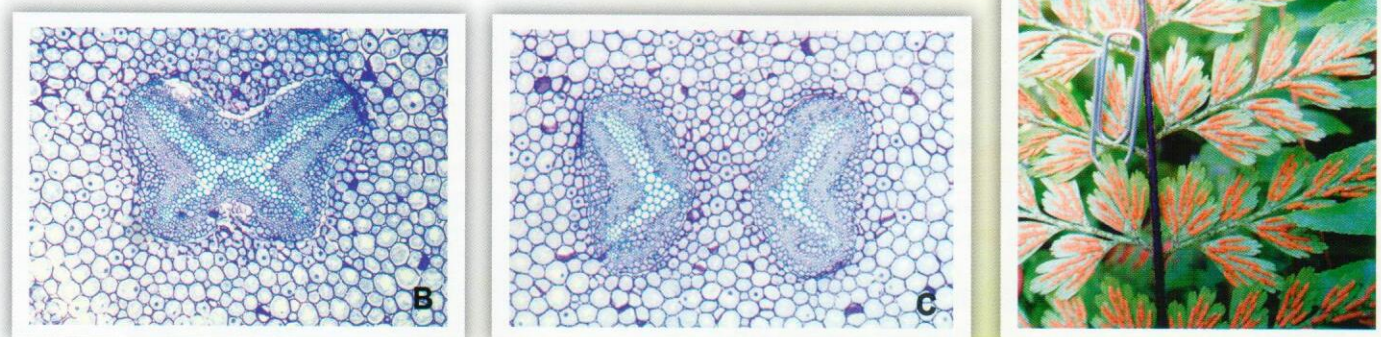


Figure 1 : *Asplenium nidus* L.

A classification of the Aspleniaceae using rhizome anatomy was attempted by Hayata (1927 and 1928). On the basis of the rhizome's radial and dorsio-ventral vascular bundles arrangement, he proposed *Asplenium*, *Boniniella* and *Hymenasplenium* for the family.



*Asplenium* was described as having radial vascular bundles whereas *Boniniella* and *Hymenasplenium* having dorsiventral vascular bundles. The separation between *Boniniella* and *Hymenasplenium* was laid on the character of vein and sori. *Boniniella* possesses reticulate veins and scolopendroid sori in comparison to free veins and linear sori for *Hymenasplenium*. However, other researchers found that morphological characters should prevail over the anatomical ones. As consequence of this disagreement, Aspleniaceae was treated as a single genus family. Copeland (1960) for example ignored the previous establishment of *Asplenium*, *Boniniella*, and *Hymenasplenium*. In addition to linear sori, he described Aspleniaceae (Figure 2) vascular bundle as having an "X" – shape at the upper petiole and two opposite "C"- shape at basal petiole. Akin to Copeland, Holttum (1968) did not recognize the genera within Aspleniaceae. Instead, he proposed the definition of species group in response to the occurrence of morphological affinities.



**Figure 2 : Character of Aspleniaceae. A. Linear sori; B. "X"-shaped vascular bundle at upper petiole; C. Two "C"-shaped vascular bundle at basal petiole.**

As for many other plant families, the absence of objective criteria for character weighting led to a disagreement on which characters genera should be distinguished. If the idea that the classification should reflect the species phylogenetic relations gained wide acceptance, the difficulty of establishing phylogenies on the basis of a small number of morphological character variations hindered further progress in plant taxonomy. This problem found a solution when techniques of DNA sequencing and computed DNA polymorphism analysis became available around 1990 and permitted to evaluate species phylogenies on the basis of the evolution of some of their genes. Molecular inference on ferns was initiated by Hasebe et al (1993, 1994, and 1995). The study indicated Aspleniaceae to be monophyletic. Later Murakini (1995) found that *Hymenasplenium* was a monophyletic group that had most basally diverged from *Asplenium*. However, the previously described distinctions between *Boniniella* and *Hymenasplenium* were not supported by the chloroplastic gene *rbcl* sequence (Murakami 1995). The first attempt to build a phylogeny of the family was conducted by Schneider et al. (2004 and 2005) using a combination of *rbcl* and *TrnL-TrnF* non coding regions onto 71 and 77 samples respectively collected from across the world. Their outcome supported many previous attempts of clarification for instance the relation of *Diellia* with the black-stemmed character of *Asplenium* suggested by Wagner (1952 and 1953a,b). Their other finding confirmed that most previously proposed genera were grouped within *Asplenium*. On the basis of these molecular studies, Smith et al. (2006)

formalized Aspleniaceae having only two genera: *Asplenium* and *Hymenasplenium*. Another study using 400 taxa by (Schuettpelz and Pryer 2007) confirmed previous findings and suggested Blechnaceae, Onoclenaceae, Thelypteridaceae, and Woodsiaceae as sister families of Aspleniaceae. In a later study using *rbcl* and *trnL-trn* region, I (Rambe 2010) used 147 taxa of Aspleniaceae collected from various phytogeographic units to correlate the inferred phylogeny with morphological observations. The outcome of this study revealed an evolutionary trend for the characters of the petiole/rachis and pinna within the Aspleniaceae. The primordial group of *Asplenium* was identified as following: rachis/petiole is circular, aerophore is in form of a developed juncture, pinna is sessile and imbricated, costa is embedded. In contrast, for the developed group: rachis/petiole is sulcate, aerophore is either vestigial or absent, pinna is stalked and free, costa is visible as sulcate or wide. This definition of evolutionary trends within the *Asplenium* permitted to reassess the character choice of previously defined species group by Holttum (1968).

For this assessment, morphological features (Figure 3) were observed on fifty three taxa of Malesian *Asplenium*, selected according to their ability to describe species groups reflecting an evolutionary trend. In comparison to the species group description made by Holttum (1968) that was mentioned to follow the species phylogeny, the outcome of the current study indicated significant differences in the character choices and achieved a higher consistency of selected characters (Table 1). In opposite



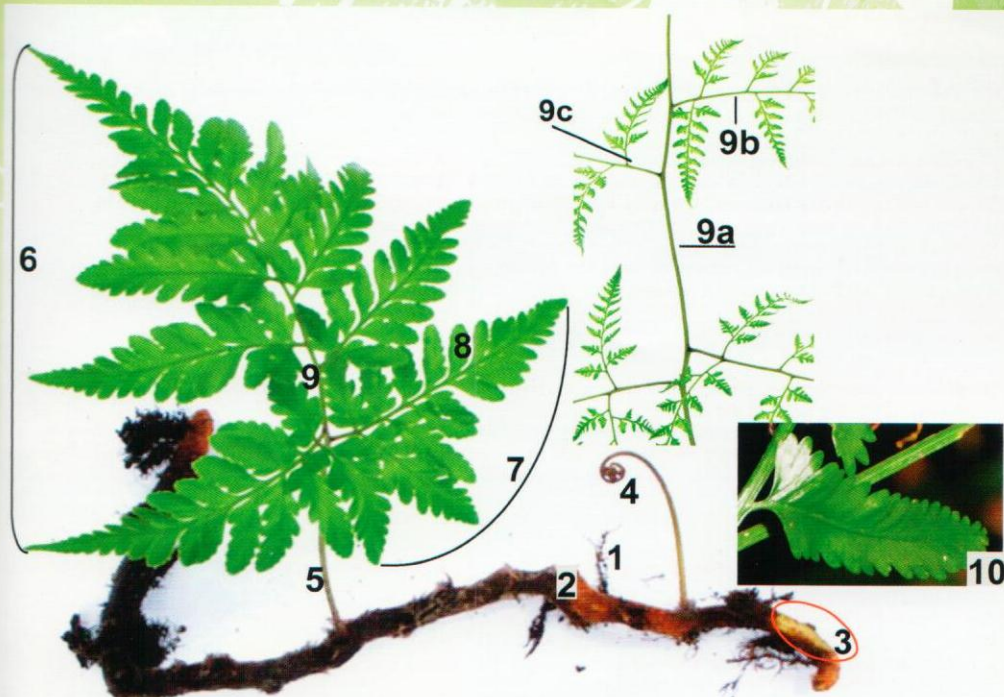


Figure 3 :

Morphology of fern. 1.Root; 2.Rhizome is covered young and old palea; 3.Rhizome tip is exposed as palea removed; 4.Crozier; 5. Petiole; 6.Frond; 7.Pinna; 8.Pinnule; 9.Rachis; 9a.primary branch; 9b. Secondary branch, 9c. tertiary branch; 10.Stalk; 11.Frond vascular bundles: 11a. rachis, 11b. costa, 11c. vein; 12. Trace of vein on the adaxial surface of pinna; 13.Costa on the adaxial surface of pinna; 14. Vestigial juncture (vj) at lateral petiole, 15.Developed juncture (dj) at the lateral sides of rachis; 16.Stomata of polocytic type; 17.India; 18.Sori; 19.Sporangium: 19a. sporangiophore, 19b. sporangium is encapsulated by annulus, 19c. stomium; 19d. spore. 20.Spores are ornamented by spiny decoration of perispore.



to the species groups of Holtum that involved micro morphological characters, the present species groups are based solely on macroscopic characters that can be linked in a logical way (Figure 4). While working with larger number of taxa, I experienced that species groups that can be identified on easily observed characters and that reflect an evolutionary trend facilitated significantly the identification of species in the field as well as the description processes of the taxa.

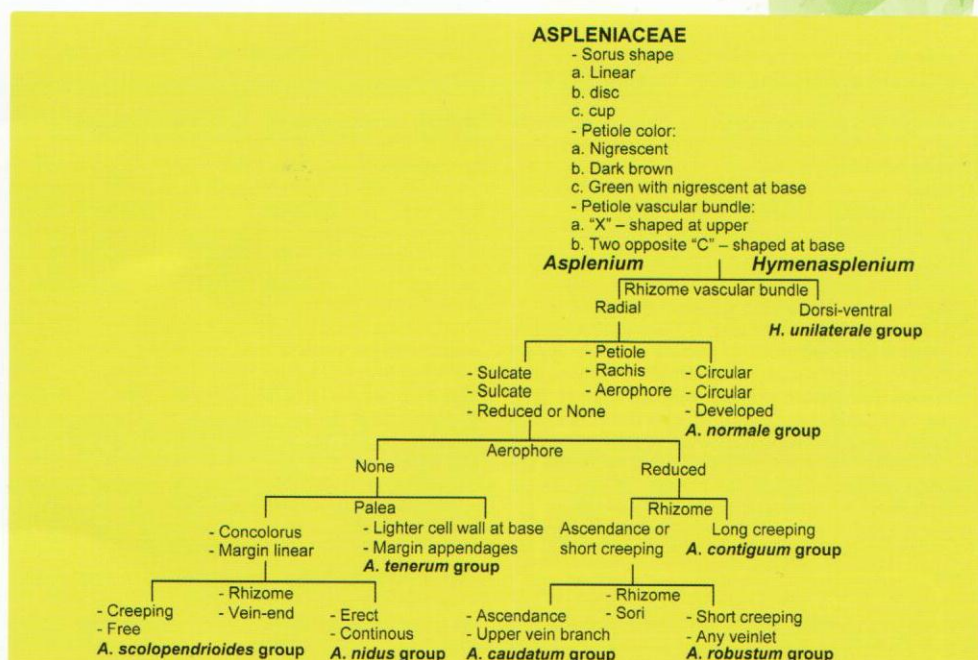


FIGURE 4. Schematic relationship within proposed species groups. Discussed of morphological characters are given in the middle of dichotomous branches. Observed variable characters are given at the terminal of dichotomous branches.



TABLE 1 : Comparison of character used between proposed groups

Holtttum (1968)	Current proposal	Evaluation
<p><b>Asplenium caudatum group:</b> Frond is mostly simple; <i>pinnae</i> with various degree of lobes; <i>spore</i> is dark, translucent, with narrower thickened wing; <i>perispore</i> is more folded in addition to the wing.</p> <p><b>Species:</b> <i>A. caudatum</i>, <i>A. adiantoides</i>, <i>A. logissimum</i>, <i>A. macrophyllum</i>, <i>A. perakense</i>, <i>A. pellucidum</i>, <i>A. spathulinum</i>, <i>A. paradoxum</i>.</p>	<p><b>Asplenium caudatum group:</b> Rhizome is ascendance; <i>petiole</i> and <i>rachis</i> are sulcate, dull, dark brown; <i>aerophore</i> is reduced; <i>frond</i> is once pinnate, <i>pinna</i> is stalked and free; <i>costa</i> is sulcate on adaxial surface and prominent on abaxial surface; <i>sori</i> is in the upper vein branch and opened towards costa.</p> <p><b>Species:</b> <i>A. approximatum</i>, <i>A. caudatum</i>, <i>A. horridum</i>, <i>A. longissimum</i>, <i>A. pellucidum</i>, <i>A. schorggessii</i>, <i>A. subavenium</i>.</p>	<p><i>A. caudatum</i> group, <i>A. contiguum</i> group and <i>A. robustum</i> group were observed having perispore which is thick fold and the surface is coarse in various degrees. Nevertheless, describing species group using micro morphology is not suggested as it is not practical during the identification.</p>
	<p><b>Asplenium contiguum group:</b> Rhizome is long creeping, densely covered by dark brown palea; <i>petiole</i> and <i>rachis</i> are sulcate; <i>aerophore</i> is reduced; <i>pinna</i> is chartaceous, stalked and free; <i>costa</i> is sulcate on adaxial surface and prominent on abaxial surface.</p> <p><b>Species:</b> <i>A. contiguum</i>, <i>A. malayo-alpinum</i>.</p>	<p>No taxa being studied by Holtttum.</p>
	<p><b>Asplenium nidus group:</b> Roots have dense root-hairs that commonly form spongy mass; Rhizome is erect; <i>palea</i> is concolorous, clathrate, and margin linear; <i>frond</i> is simple; <i>lamina</i> is coriaceous, apex not proliferous; <i>vein</i> ends are connected and form marginal vein; <i>costa</i> is wide; <i>indusium</i> is reflexed at maturity.</p> <p><b>Species:</b> <i>A. australasicum</i>, <i>A. curtisorum</i>, <i>A. cymbifolium</i>, <i>A. grevillei</i>, <i>A. musifolium</i>, <i>A. nidus</i>, <i>A. phyllitidis</i>.</p>	<p>Not recognized by Holtttum.</p>
	<p><b>Asplenium normale group:</b> Rhizome is erect; <i>petiole</i> and <i>rachis</i> is circular, glabrescent, shiny, nigrescent; <i>aerophore</i> is developed, <i>pinna</i> is sessile; <i>costa</i> is embedded.</p> <p><b>Species:</b> <i>A. minus</i>, <i>A. normale</i>.</p>	<p>Not recognized by Holtttum.</p>
<p><b>Asplenium robustum group:</b> Frond is fairly tough; <i>pinnae</i> is finely dissected; <i>epiphyte</i>; <i>costa</i> with a small ridge; <i>spore</i> is pale, translucent; <i>perispore</i> is rather broad, almost entire wing and has few folds.</p> <p><b>Species:</b> <i>A. robustum</i>, <i>A. glaucophyllum</i>, <i>A. sublaserpitiifolium</i>.</p>	<p><b>Asplenium robustum group:</b> Rhizome is short creeping; <i>petiole</i> and <i>rachis</i> are sulcate; <i>aerophore</i> is reduced; <i>pinna</i> is stalked and free; <i>costa</i> is sulcate on adaxial surface and prominent on abaxial surface; <i>sori</i> are situated at veinlet.</p> <p><b>Species:</b> <i>A. adiantoides</i>, <i>A. amaurobulum</i>, <i>A. laserpitiifolium</i>, <i>A. macrophyllum</i>, <i>A. nitidum</i>, <i>A. perakense</i>, <i>A. praemorsum</i>, <i>A. rockii</i>, <i>A. robustum</i>, <i>A. spathulinum</i>.</p>	<p><i>A. robustum</i> group is also composed by once pinnate frond. The principle comparisons to <i>A. caudatum</i> group are: <i>sori</i> occur at vein-let where the indusium is opened towards the adjacent vein and <i>sori</i> occur at upper vein branch where indusium opens towards costa.</p>
<p><b>Asplenium scolopendroides group:</b> Rhizome is creeping; <i>frond</i> is rather narrow, moderate size, simple frond or simple pinnate; <i>sori</i> of the adjacent vein are often face each other; <i>spores</i> very distinctive; <i>perispore</i> is pale with spine-like.</p> <p><b>Species:</b> <i>A. scolopendroides</i>, <i>A. borneense</i>, <i>A. batuense</i>, <i>A. scalare</i>, <i>A. nidus</i>, <i>A. phyllitidis</i>.</p>	<p><b>Asplenium scolopendroides group:</b> Rhizome is creeping; <i>palea</i> is concolorous, opaque and, margin linear; <i>petiole</i> and <i>rachis</i> is sulcate, glabrate, green, dull; no <i>aerophore</i>; <i>pinna</i> is stalked; <i>costa</i> is wide; <i>vein</i> is free. The character of <i>petiole</i> and <i>rachis</i> are not applicable for the simple frond.</p> <p><b>Species:</b> <i>A. acustiusculum</i>, <i>A. amboinense</i>, <i>A. batuense</i>, <i>A. borneense</i>, <i>A. paradoxum</i>, <i>A. scalare</i>, <i>A. scolopendroides</i>, <i>A. simplex</i>, <i>A. spathulatum</i>, <i>A. subscalare</i>, <i>A. taeniosum</i>.</p>	<p>The scolopendroid <i>sori</i> occur occasionally. The most remarkable characters for this group within the simple frond are: rhizome is long creeping; vein is free, simple or once forked.</p>
<p><b>Asplenium tenerum group:</b> Rhizome is erect, apex covered with brown palea, bears rosette arrangement of <i>petiole</i>; <i>petiole</i> is light green; <i>frond</i> is simple or simple pinnate; <i>costa</i> broad, rise up on adaxial margin <i>pinnae</i> dentate; <i>indusim</i> is round and pale; <i>spore</i> is pale, opaque, granular, wing with a minutely toothed, thickened edge, moderate width; <i>perispore</i> is anastomosing folded.</p> <p><b>Species:</b> <i>A. tenerum</i>, <i>A. belangeri</i>, <i>A. scortechinii</i>, <i>A. salignum</i>, <i>A. squamulatum</i>, <i>A. nidus</i>, <i>A. phyllitidis</i>.</p>	<p><b>Asplenium tenerum group:</b> Rhizome is erect; <i>palea</i> is clathrate, lighter color of cell wall at base, margin appendages; <i>petiole</i> and <i>rachis</i> is sulcate, glabrescent, shiny; no <i>aerophore</i>; <i>pinna</i> is stalked and free; <i>costa</i> is raised on both adaxial and abaxial surface; <i>vein</i> is free; <i>indusium</i> is reflexed at maturity. The character of <i>petiole</i> and <i>rachis</i> are not applicable for the simple frond.</p> <p><b>Species:</b> <i>A. belangeri</i>, <i>A. prolongatum</i>, <i>A. salignum</i>, <i>A. scortechinii</i>, <i>A. squamulatum</i>, <i>A. tenerum</i>, <i>A. vulcanicum</i>.</p>	<p><i>A. tenerum</i> group is separated from the rest of species group on the basis of <i>palea</i> character which the cell wall is lighter color at base and margin is appendages. Within the simple frond, <i>A. tenerum</i> group is identified from <i>A. nidus</i> by having free vein and from <i>A. scolopendroides</i> by having erect rhizome.</p>
<p><b>Asplenium unilaterale group:</b> Rhizome is creeping; <i>spore</i> is dark with wide transparent wing, edge is uneven, often toothed; <i>perispore</i> is anastomosing folded.</p> <p><b>Species:</b> <i>A. chelisorum</i>, <i>A. excisum</i>, <i>A. normale</i>, <i>A. subnormale</i>, <i>A. unilaterale</i>.</p>	<p><b>Hymenasplenium unilaterale group:</b> Rhizome is long creeping; <i>petiole</i> is glabrescent.</p> <p><b>Species:</b> <i>A. apogamum</i>, <i>A. cheilosorum</i>, <i>A. excisum</i>, <i>A. obscurum</i>, <i>A. subnormale</i>, <i>A. unilaterale</i>.</p>	<p>Dorsi-ventral vascular bundle is character for <i>Hymenasplenium</i>. Character of genus should not be applied to define the species group. Nonetheless, <i>A. normale</i> is species member of <i>Asplenium</i>.</p>



## REFERENCES

- Adanson, M. 1763. Familles des plantes. Chez Vincent. Imprimeur – libraire de Mgr Le comte de Provence, rue S. Severin, Paris.
- Copeland, E.B. 1960. Ferns flora of the Philippines. Monograph Vol. 3. 429-453. Manila Bureau of Printing, Philippines.
- Frank, A.B. 1877. Synopsis der pflanzenkunde. Dritte abtheilung. Cryptogamae L. Die Kryptogamen, verborgenblütige pflanzen, sporen. In Leunis, J. (ed.): Synopsis der pflanzenkunde [...]. 1231-1961. Hahn'sche Buchhandlung, Hannover.
- Hasebe, M., Ito, M., Kofuji, R., Ueda, K., Iwatsuki, K. 1993. Phylogenetic relationship of ferns deduced from rbcL gene sequences. J. Mol. Evol. 37: 476-482.
- Hasebe, M., Otori, T., Nakazawa, M., Sano, T., Kato, M., Iwatsuki, K. 1994. rbcL gene sequences provide evidence for the evolutionary lineage of leptosporangiate ferns. Proc. Natl. Acad. Sci. USA 91: 5730-5734.
- Hasebe, M., Wolf, P.G., Pryer, K.M., Ueda, K., Ito, M., Sano, R., Gastony, J.G., Yokoyama, J., Manhart, R.J., Murakami, N., Crane E.H., Haufler, C.H., Hauk, W.D. 1995. Ferns phylogeny based on rbcL nucleotide sequences. Amer. Ferns J. 85: 134-181.
- Hayata, B. 1927. On the systematic importance of the steler system in the filicales. 1. Bot. Mag. Tokyo 41: 697-718.
- Hayata, B. 1928. On the systematic importance of the steler system in the Filicales III. Bot. Mag. Tokyo 42: 334-348.
- Holttum, R.E. 1968. Ferns: A Revised Flora of Malaya. Vol. II. Government Printing, Singapore.
- Johns, R.J. 1996. A field study of Asplenium Sect. Thamnopteris in the N.E. Kepala Burung, Irian Jaya, Indonesia. In Camus, J.M., Gibby, M., Johns, R.J., (eds.): Pteridology in Perspective: 337-340. Royal Botanic gardens, Kew.
- Khare, P.K., and Shankar, R. 1987. On the petiolar structure of some Asplenium species. Can. J. Bot. 7: 95-103.
- Kramer, K.U., and Viane, R.L.L. 1990. Aspleniaceae. In Kramer, K.U. and Green P.S., (Eds.): The family and genera of vascular plants Vol. 1; Pteridophytes and Gymnosperm: 52-57. Springer Verlag, Berlin.
- Linnæi, C. 1738. Classes Plantarum. Conradum Wishoff, Lugduni Batavorum.
- Linnæi, C. 1753. Species Plantarum, Exhibentes Plantas Rite Cognitas ad Genera Relatas, cum Differentiis Specificis, Nominibus Trivialibus, Synonymis Selectis, Locis Natalibus, Secundum Systema Sexuale Digestas, Tomus II. L. Salvius, Stockholm.
- Murakami, N. 1995. Systematics and evolutionary biology of the fern genus Hymenasplenium (Aspleniaceae). J. Plant Res. 108 (2): 257-268.
- Rambe, S.K. 2002. Taxonomic and phytogeographic studies on Aspleniaceae in Peninsular Malaysia, Sumatra and Java. M.Sc. thesis. Nanyang Technological University.
- Rambe, S.K. 2010. Phylogeny analysis of the Aspleniaceae: an assessment of its taxonomy and distribution. Ph.D thesis. Nanyang Technological University, Singapore.
- Ruellio, J. 1539. Pedanii Dioscoridis Anazarbei De Medica Materia Libri Sex. Apud Mich. Insignirum, Basileæ.
- Schneider, G. 1892. The Book of Choice Ferns. Vol. I. L. Upcott Gill, London.
- Schneider, H., Russell, J., Cox, C.J., Bakker, F., Henderson, S., Rumsey, F., Barret, J., Gibby, M., Vogel, J.C. 2004. Chloroplast phylogeny of Asplenoid ferns based on rbcL and TrnL-F spacer sequences (polypodiidae, Aspleniaceae) and its implications for biogeography. Syst. Bot. 29 (2): 260-274.
- Schneider, H., Ranker, T.A., Russell, S.J., Cranfill, R., Geiger, J.M.O., Aguraju, R., Wood, K.R., Grundmann, M., Kloberdanz, K., Vogel, J.C. 2005. Origin of the endemic ferns genus Diellia coincided with the renewal Hawaiian terrestrial life in the Miocene. Proc. R. Soc. Biol. 272: 455-460.
- Schuettpelz, E., and Pryer, K.M. 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. Taxon 56(4): 1037-7E(1029).
- Smith, A.R. Pryer, K.M., Schuettpelz, E., Korall, P., Schneider, H., Wolf, P.G. 2006. A classification for extant taxa. Taxon 55(3): 705-731.
- Wagner Jr, W. H. 1952 The fern genus Diellia, its structure, affinities, and taxonomy. Univ. Calif. Publ. Bot. 26: 1-212.
- Wagner Jr, W. H. 1953a An Asplenium prototype of the genus Diellia. Bull. Torrey Bot. Club 80: 76-94.
- Wagner Jr, W. H. 1953b The genus Diellia and the value of characters in determining fern affinities. Amer. J. Bot. 40: 34-40.



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