UNIVERSITI PUTRA MALAYSIA

MORPHOLOGY, ANATOMY AND MOLECULAR CHARACTERISTICS OF BLECHNACEAE FERNS FROM PENINSULAR MALAYSIA

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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August 2014

Chairman: Professor Umi Kalsom Binti Yusuf, PhD

Faculty: Science

The arguments and unclear status upon Blechnaceae taxonomic classification which considered as monophyletic by several authors but showing different results to others has growing interest to study this family. Blechnaceae from Peninsular Malaysia which represented by four genera; *Blechnum*, *Woodwardia*, *Brainea* and *Stenochlaena* were studied. These ferns were distributed and collected from three different states namely Pahang, Johor and Selangor. The taxonomic placement of this ferns at ordinal and familial was categorized differently among previous taxonomist. This may confound present access and brought difficulty to identify the plants. Furthermore, molecular data previously has also resulted with two-differently species relation among Blechnaceae; (1) monophyletic, and (2) not monophyletic. These findings have brought intense interest to study ferns of Blechnaceae with objectives to document the macro morphological characters including rhizome, frond architecture, venation and scales. Each character was observed and identified upon similarities and differences with aid of magnification. The sectioned of stipe and rachis from fresh specimens were done to study vascular bundle arrangement. Meanwhile, to classify this family using molecular approaches, chloroplast coding and non-coding regions of *rbcL*, *atpB* and *trnH-psbA* were amplified using Polymerase Chain Reaction (PCR) and the sequences were analyzed to indicate species relatedness. All data were compiled using NCSS[9] Statistical Software and Graphic Analysis and further analyzed using the Unweighted-Pair Group method. The results of the study showed Blechnaceae

is not monophyletic. *Blechnum* was found to be clustered into two clades, while *Brainea* and *Woodwardia* were treated as the ancestors to the family. *Stenochlaena* was found closely related to *B. indicum*, whereas a new species of *Blechnum* sp. was relatively close and much resembled *B. orientale*. The result obtained from clustering analysis was supported by the Maximum Parsimony analysis using the nucleotide sequences from *rbcL*, *atpB* and *trnH-psbA*. *RbcL* and *trnH-psbA* were found as two reliable markers in this study due to its parallel hypothesis in inferring the species relatedness. However, *atpB* sequences were too short and could be unreliable to resolve the species relationship. The classification by clustering analyses using macro and micro morphological characters were unparallel with the molecular finding. All species were found as not monophyletic and but able to be distinguished separately. Each species had their own characters but some of them shared with overly similarly characters. However, the data from this study could be use for future references when conducting research of ferns from Peninsular Malaysia.

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CIRI-CIRI MORFOLOGI, ANATOMI DAN MOLEKUL PAKU PAKIS BLECHNACEAE DARI SEMENANJUNG MALAYSIA

Oleh

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Perbezaan pendapat dan status yang tidak jelas terhadap pengkelasan taksonomi famili Blechnaceae yang dikatakan sebagai monofiletik oleh sesetengah pengkaji tetapi bukan bagi sebahagian pengkaji yang lain mengukuhkan lagi tujuan kajian ini dilakukan. Paku pakis Blechnaceae dari Semenanjung Malaysia yang diwakili oleh empat genera; *Blechnum*, *Woodwardia*, *Brainea* dan *Stenochlaena* telah dikaji. Paku pakis tersebut boleh didapati dari tiga negeri yang berbeza iaitu Pahang, Johor dan Selangor. Kedudukan taksonomi paku pakis ini dikategorikan secara tidak selari pada peringkat order dan famili oleh pengkaji-pengkaji terdahulu. Hal ini menyebabkan kesulitan untuk mengakses dan mengenalpasti tumbuhan ini. Tambahan pula, data dari aspek molekular juga telah menunjukkan spesies dari famili Blechnaceae dikelaskan kepada dua; (1) monofiletik, dan (2) bukan monofiletik. Dapatan kajian tersebut meningkatkan lagi rasa minat dan perlunya kajian terhadap famili Blechnaceae dilakukan. Objektif kajian ialah merekodkan ciri-ciri makro morfologi seperti rizom, susunan daun, corak urat daun dan rerambut. Ciri-ciri morfologi tersebut dikenalpasti persamaan dan perbezaannya dengan bantuan kanta pembesar. Manakala, ciri-ciri mikro morfologi berkas vaskular dari bahagian batang iaitu stip dan rakis juga dilakukan menggunakan teknik hirisan menggunakan mikroskop gelongsor. Bagi mengkategorikan paku pakis ini dengan lebih tepat, pendekatan molekul menggunakan teknik "Polymerase Chain Reaction (PCR) dijalankan. Kesemua jujukan genetik yang diperolehi digabungkan dan dianalisa untuk menentukan hubungan antara spesies yang

dikaji. Data yang diperolehi dari aspek morfologi dan genetik digabungkan dan dianalisis menggunakan perincian "NCSS[9] Statistical Software and Graphic Analysis" di mana analisis pengkelasan menggunakan kaedah "Unweighted-Pair Group" dilakukan. Hasil kajian mendapati paku pakis Blechnaceae adalah bukan monofiletik. Genus *Blechnum* didapati membentuk dua kumpulan berdasarkan persamaan yang ditunjukkan oleh ciri-ciri morfologi dan disokong oleh data molekul. Tambahan pula, *Blechnum* didapati berasal dan mempunyai perkaitan dengan dua induk iaitu *Brainea* dan *Woodwardia.* Manakala, *S. palustris dan B. indicum* mempunyai perkaitan yang rapat berdasarkan data dari *rbcL* dan juga *trnH-psbA*. *Blechnum* sp. diklasifikasi sebagai spesies yang mempunyai perkaitan rapat dengan *B. orientale* sama ada dari aspek morfologi mahupun molekul. Kajian ini juga telah menunjukkan data daripada aspek makro morfologi dan mikro morfologi adalah tidak selari dengan data dari pepohon molekul. Berdasarkan kepada dapatan kajian molekul, penanda molekular *rbcL* dan *trnH-psbA* merupakan dua penanda yang boleh dipercayai dan menghasilkan keputusan analisis yang selari. Walau bagaimanapun, penanda molekular *atpB* tidak menunjukkan hasil analisis yang baik, memandangkan jujukannya yang pendek dan tidak menghasilkan data yang mencukupi. Oleh yang demikian, penanda molekular *atpB* ini didapati bukan penanda yang baik bagi kajian ini. Secara keseluruhannya, dapatan kajian ini mendapati paku pakis dari famili Blechnaceae adalah bukan monofiletik dan data yang diperolehi dari kedua-dua skop iaitu morfologi dan molekul boleh digunakan untuk kajian masa depan dan menjadi rujukan bagi paku pakis di Semenanjung Malaysia.

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CHAPTER 1

INTRODUCTION

A recircumscription of Blechnaceae family based on both morphological and molecular data has resulted with ca. nine genera; *Blechnum* L., *Brainea* J. Sm., *Doodia* R. Br., *Pteridoblechnum* Hennipman, *Sadleria* Kaulf., *Salpichlaena* J. Sm., *Steenisioblechnum* Hennipman, *Stenochlaena* J. Sm., and *Woodwardia* Sm. (Smith *et al*., 2006a). However, only four genera were found in Peninsular Malaysia which are *Blechnum*, *Brainea*, *Woodwardia,* and *Stenochlaena*. *Brainea* and *Woodwardia* are treated as old genera from which *Blechnum* is derived while *Stenochlaena* is a sole climbing fern in the family (Holttum, 1968; Piggottt, 1988).

Blechnaceae from Peninsular Malaysia comprises of nine species; *Blechnum orientale*, *Blechnum finlaysonianum*, *Blechnum indicum*, *Blechnum fraseri*, *Blechnum melanocaulon* subsp. *pallens*, *Blechnum vestitum*, *Stenochlaena palustris*, *Woodwardia auriculata* and *Brainea insignis*. The distribution pattern of Blechnaceae family is various; some of them live in highland areas of Cameron Highlands in Pahang with high humidity (e.g. *B. fraseri*, *B. vestitum*, *B. melanocaulon* subsp. *pallens,* and *W. auriculata*); *Br. insignis* was found in offshore islands of Mersing, Johor; *B. indicum* in swamp areas of Mersing, Johor; and *B. orientale* and *S. palustris* are found abundantly in sun-exposed areas. However, species of *B. finlaysonianum* requires moist and shaded environment and it was found in Selangor. In this study, a new sample has been found in mountainous areas of Fraser's Hill in Pahang, and namely *Blechnum* sp. This species is still under research on its relationship towards the Blechnaceae family.

Ferns usually have a long history in Chinese medicinal usage. They had abundant of chemical compounds with pharmacological properties (Xin *et al*., 2010). *Blechnum* possess ethnomedicinally properties, for treatment of various skin diseases, stomach pain, urinary bladder infections and sterilization in women. *B. orientale*, in this case was proven as a potential candidate with antioxidant properties, for colon cancer therapy and can be used to treat Gram positive bacterial infections (Lai *et al*., 2010). Some of ferns from this family like *S. palustris* and *B. orientale* are edible. Usually, the young leaf is eaten raw. The leaves of *S. palustris* is also used to treat fever (Mannan *et al*., 2008), meanwhile *Woodwardia* and *B. orientale* were found with medicinal values (Xin *et al*., 2010) and it is important to identify these species correctly, especially those with medicinal potentials. To identify these functional species, morphological authentication is inadequate; it was confronted with difficulties with overly

similarly traits used for taxonomic characterization, and decreasing number of expertise (Xin *et al*., 2010).

The previous classification of Blechnaceae by Holttum (1968) has no agreement in terms of familial and ordinal placement with other taxonomists such as Beddome (1969) and Tryon and Tryon (1982). Holttum (1968) classified Blechnaceae with three genera as Blechnoidea, a sub-family of Dennstaedtiaceae which are (1) *Blechnum*, which comprises of *B. orientale*, *B. finlaysonianum*, *B. indicum*, and *B. vestitum*; (2) *W. auriculata*; and (3) *Br. insignis.* The researcher also recategorized *S. palustris* into Pteridoideae, a sub-family of Aspleniaceae. The dismemberment of *S. palustris* is due to its affinities to *Acrostichum* in the structure of vascular system of rhizome and stipe, reduced pinnae bearing glands, and colourless translucent spores without perispores.

Beddome (1969), on the other hand, classified *Blechnum* and *Woodwardia* into Polypodiaceae, *Br. insignis* in the tribe of Grammitidea, and *S. palustris* which was previously named as *S. palustre* in the tribe of Acrosticheae, by characterizing the dissimilarities in their spore structure and localization. His classification conveys significant distinction from Holttum's.

Meanwhile, Tryon and Tryon (1982) have indicated three genera in Blechnaceae; (1) *Salpichlaena*, (2) *Blechnum,* and (3) *Woodwardia*. They found Blechnaceae as a distinctive family by having an unfolded lamina which is tinged in red. However, these ferns resemble more to Aspleniaceae in terms of their sorus, indusium, and chromosome number (n= 36).

Based on the similarities in morphological characters such as sori distribution which aligns along costa or costule, nine species of Blechnaceae have been clustered into a single family by Piggott (1988). The nine species are *B. orientale*, *B. finlaysonianum*, *B. indicum*, *B. fraseri*, *B. vestitum*, *B. patersonii*, *W. auriculata*, *S. palustris,* and *Br. insignis*.

The distinctions in the classification of Blechnaceae result in equivocal taxonomic placement and confusion in the family recognition. In addition, the morphological characters which have been used as taxonomic tools to identify certain species are somewhat highly related with each other without significant difference.

Thus, when molecular data for Blechnaceae has been introduced by Smith *et al*. (2006), Schuettpelz and Pryer (2007), and Shepherd *et al*. (2007), it provides complementary information for the previous classification which was based on morphological authentication only. However, the intention in molecular study was to determine the monophyly of the family using several molecular markers, mostly from chloroplast, mitochondria, and nuclear regions. The monophyletic relationship shows a common ancestral of the family and can be shown when a species is grouped together by forming a clade. The overlapping of different species and genera within a clade shows a paraphyletic relationship.

The progress in molecular studies results in Blechnaceae being considered as monophyletic (Smith *et al*., 2006b; Hasebe *et al*., 1995). Nevertheless, studies by Cranfill (2001), Schuettpelz and Pryer (2006), and Shepherd *et al*. (2007) have proved that they are not monophyletic since more than one genus are nested within *Blechnum*. The research findings according to molecular data are clearly unlike the classical characterization, which mainly based on morphological characters. These arguments and unparallel classifications of Blechnaceae from both morphological and molecular approaches give a significant and strong reason to study ferns of Blechnaceae from Peninsular Malaysia. Furthermore, the growing interest in molecular approach among biologists nowadays increases the reliability of taxonomic classification by consuming various data from the plants.

Hence, the objectives of this study are (1) to document macro morphological characters such as rhizome, frond architecture, venation, scales and micro morphological character of vascular bundle from the stipe and rachis from Blechnaceae and the data will be analyze using Group Average (Unweighted-Pair Group) clustering analysis to infer species relatedness; (2) to develop the data from *rbcL*, *atpB* and *trnH-psbA* regions as identification tools to support or disapprove the existing classifications of Blechnaceae from Peninsular Malaysia.

REFERENCES

Armstrong, W.P. (2002). Wayne's Word. http://waynesword.palomar.edu/index.htm. Retrieved 2 November 2013.

Australian Plant Society (2011). Topic 8: Ferns produced by North Shore Group. http://plantnet.rbgsyd.nsw.gov.au/. Retrieved15 May 2013.

Beddome, R.H. (1969). *Handbook to the ferns of british india ceylon and the malay peninsula with supplement*. New Delhi, India: Today & Tomorrow's Printers & Publishers.

Berlyn, G.P & Miksche, J.P. (1976). Botanical microtechnique and cytochemistry. Ames, Iowa: The Iowa State University Press, pp.326

Bousquet, J., Strauss, S.H., Doerksen, A.H. & Price, R.A. (1992). Proceeding from the National Academy of Science 89: *Extensive variation in evolutionary rate of rbcL gene sequences among seed plants*, 7844-7848, USA.

Bowman, S.M., Patel, M., Yerramsetty, P., Mure, C.M., Zielinski, A.M., Bruenn, J.A. & Berry, J.O. (2013). A novel RNA binding protein affects *rbcL* gene expression and is specific to bundle sheath chloroplasts in C4 plants. *Biomed Central Plant Biology*, 13: 138.

Brandon, S.G., Lynn, G.C., Jonathan, F.W. & Spencer, V.M. (1997). Comparisons of the molecular evolutionary process at *rbcL* and *ndhF* in the grass family (Poaceae). *Molecular Biology Evolution*, *14*(7): 769-777.

Carr, S. M. (2002). Principles of parsimony analysis: an example with molecular data. http://www.mun.ca/biology/scarr/2900_Parsimony_Analysis.htm. Retrieved 5 May 2013.

CBOL Plant Working Group (2009). Proceedings from the National Academy of Sciences of the United States of America 106 (31): *A dna barcode for land plants*, 12794-12797. Doi: 10.1073/pnas.0905845106

Chambers, T.C. & Farrant, P.A. (2001). Revision of Blechnum (Blechnaceae) in Malesia. *Blumea*, 46: 283-350.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., & Leon, C., (2010). "Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species", *PLoSone, 5*(1):1–8. Doi: 10.1371/journal.pone.0008613.

Clegg, M.T. & Zurawski, G. (1991). Chloroplast DNA and the study of plant phylogeny. In Soltis, P.S., Soltis, D.E. & Doyle, J.J. (eds.), *Molecular Systematics of Plants*, 1 – 13. New York: Chapman and Hall.

Cranfill, R. (2001*). Phylogenetic studies in the Polypodiales (Pteridophyta) with an emphasis on the family Blechnaceae*. Ph.D. thesis, University of California, Berkeley, California.

Downie, S. R. & Palmer, J.D. *(*1992). Use of chloroplast DNA rearrangements in reconstructing plant phylogeny. In Soltis, P.S., Soltis, D.E. and Doyle, J.J. (eds.), *Molecular Systematics of Plants*, 14-35. New York: Chapman and Hall.

Doyle, J.J. & Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantity of fresh leaf tissue. *Phytochemistry Bulletin*, 19: 11 – 15.

Dunlap, M., & Adaskaveg, J. E. (1997). Introduction to the Scanning Electron Microscope. *Theory, practice, & procedures. Facility for Advance Instrumentation. UC Davis*, pp.52.

Earle, S. C. Jr., (1971). Preparing herbarium specimens of vascular plants. *U.S.D.A. Agriculture Information Bulletin*, 348, Washington, D.C. http://www.ethnoleaflets.com/prepare.htm. Retrieved 2 August 2012.

Eckert, K.A., & Kunkel, T.A. (1991). DNA polymerase fidelity and the polymerase chain reaction. *PCR Methods and Applications*, *1*(1): 17-24.

Fazekas, A. J., Burgess, K. S., Kesanakurti, P. R., Graham, S. W., Newmaster, S., Husband, B. C., & Barrett, S. C. (2008). Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well. *PLoSone*, *3*(7). Doi: 0.1371/journal.pone.0002802.

Hamby, R. K., & Zimmer, E. A. (1992). Ribosomal RNA as a phylogenetic tool in plant systematics. In *Molecular systematics of plants*, 50-91.USA: Springer.

Harrington, H.D. (1957). *How to identify plants*. Ohio, US: Swallow Press.

Hasebe, M., Ito, M., Kofuji, R., Ueda, K., & Iwatsuki, K. (1993). Phylogenetic relationships of ferns deduced from *rbcL* gene sequence. *Journal of molecular evolution*, *37*(5): 476-482.

Hasebe, M., Omori, T., Nakazawa, M., Sano, T., Kato, M., & Iwatsuki, K. (Eds.) (1994). Proceedings from the National Academy of Sciences '91(12): *rbcL gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns.* USA.

Hasebe, M., Wolf, P. G., Pryer, K. M., Ueda, K., Ito, M., Sano, R., & Hauk, W. D. (1995). Fern phylogeny based on *rbcL* nucleotide sequences. *American Fern Journal*, 134-181.

Hickey, L.J. (1973). Classification of the architechture of dicotyledonous leaves. *American Journal of Botany*, *60*(1): 17-33.

Hodge, W.H. (1947). The use of alcohol in plant collecting. *Rhodora*, 49: 207 – 210.

Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., van der Bank, M., & Wilkinson, M. J. (2009). Proceedings of the National Academy of Sciences, *106*(31). *A DNA barcode for land plants*, 12794-12797.

Holttum, R.E. (1968). *A revised flora of Malaya*: *Ferns of Malaya.* Singapore: Government Printing Office, pp.443-451; 410-413.

Huang, Y. L., & Shi, S. H. (2002). Phylogenetics of Lythraceae sensu lato: a preliminary analysis based on chloroplast *rbcL* gene, *psaA-ycf3* spacer, and nuclear rDNA internal transcribed spacer (ITS) sequences. *International journal of plant sciences*, *163*(2): 215-225.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide. *Journal of molecular evolution*, *16*(2): 111-120.

Korall, P., Pryer, K. M., Metzgar, J. S., Schneider, H., & Conant, D. S. (2006). Tree ferns: monophyletic groups and their relationships as revealed by four protein-coding plastid loci. *Molecular Phylogenetics and Evolution*, *39*(3): 830-845.

Kress, W.J. & Erickson, D.L. (2007). A two-locus global DNA barcode for land plants: the coding *rbcL* gene complements the non-coding *trnH-psbA* spacer region. *PLoSone, 2*(6). Doi: 10.1371/journal.pone.0000508.

Kuo, L. Y., Li, F. W., Chiou, W. L., & Wang, C. N. (2011). First insights into fern *mat*K phylogeny. *Molecular Phylogenetics and Evolution*, *59*(3): 556-566.

Lellinger, D.B. (1985). *A field manual of the ferns and ferns-allies of the United States and Canada*. Washington, DC: Smithsonian Institution Press ix.

Lellinger, D.B. (2002). *A modern multilingual glossary for taxonomic pteridology*. American Fern Society: Missouri Botanical Garden Press.

Lipscomb, D. (1998). *Basics of Cladistic Analysis*. Student guide makalah. George Washington University. http://www.gwu.edu/~clade/faculty/lipscomb/Cladistics.pdf. Retrieved 25 September 2013.

Liu, H. M., Zhang, X. C., Wang, W., Qiu, Y. L., & Chen, Z. D. (2007). Molecular phylogeny of the fern family Dryopteridaceae inferred from chloroplast *rbcL* and *atpB* genes. *International Journal of Plant Sciences*, *168*(9): 1311-1323.

Ma, X.Y., Xie, C.X., Liu, C., Song, J.Y., Yao, H., Luo, K., & Chen, S.L. (2010). Species identification of medicinal pteridophytes by a DNA barcode marker, the chloroplast *psbA-trnH* intergenic region. *Biological and Pharmaceutical Bulletin*, *33*(11): 1919-1924.

Mader, S. (2010).*Biology 10th edition*. USA: McGraw-Hill Higher Education.

Mannan, M.M., Maridass, M. & Victor, B. (2008). A review on the potential uses of ferns. *Ethnobotanical Leaflets*, 12: 281-285.

Mehra, P. N. & Chopra, N. (1951). The anatomy of Stenochlaena palustris (Burm.) Bedd. *Annals of Botany*, *15*(1): 37-46.

Metcalfe, C.R. (1968). Current developments in systematic plant anatomy. *In Modern methods in Plant Taxonomy*, edited by Heywood, V.H., 45-57.

Meyer, F.J. (1935). Untersuchungen an den leitbundelsystemen der Alismataceenblatter als Beitrage Zur Kewnntnis der Bedingtheit und der Leistungen der Leitbundleverbindungen. *Planta*, 23: 557-592.

Newmaster, S.G., Fazekas, A.J., Steeves, R.A.D. & Janovec, J. (2008). Testing candidate plant barcode regions in the Myristicaceae. *Molecular Ecology Resources*, 9: 172-180.

Palmer, J. D. (1992). Mitochondrial DNA in plant systematics: applications and limitations. In *Molecular systematics of plants*, 36-49.USA: Springer.

Piggott, A.G. (1988). *Ferns of Malaysia in color*. Kuala Lumpur: Tropical Press Sdn. Bhd.

Pryer, K. M., Schuettpelz, E., Wolf, P. G., Schneider, H., Smith, A. R., & Cranfill, R. (2004). Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. *American Journal of Botany*, *91*(10): 1582-1598.

Rickard, M. (2005). *Gardening with ferns*. Boston, Massachusett: Holticulture Publications.

Rothfels, C. J., Sundue, M. A., Kuo, L. Y., Larsson, A., Kato, M., Schuettpelz, E., & Pryer, K. M. (2012). A revised family-level classification for eupolypod II ferns (Polypodiidae:Polypodiales). *Taxon*, *61*(3): 515-533.

Royal Botanic Gardens Melbourne (2010). Preparing herbarium specimens. http://www.kew.org. Retrieved 4 January 2013.

Saitou, N., & Imanishi, T. (1989). Relative efficiencies of the Fitch-Margoliash, maximum-parsimony, maximum-likelihood, minimum-evolution, and neighbor-joining methods of phylogenetic tree construction in obtaining the correct tree. *Molecular biology and evol*ution, *6*(5): 514-525.

Sang, T. (2002). Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical reviews in biochemistry and molecular biology*, *37*(3): 121-147.

Schuettpelz, E. R. I. C., & Pryer, K. M. (2008). Fern phylogeny. *Biology and evolution of ferns and lycophytes*, 395-416.

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

Schuettpelz, E., Korall, P. & Pryer, K.M. (2006). Plastid *atpA* data provide improved support for deep relationships among ferns. *Taxon*, 55(4): 897 – 906.

Shepherd, L.D., Perrie, L.R., Parris, B.S., & Brownsey, P.J. (2007). A molecular phylogeny for the New Zealand Blechnaceae ferns from analyses of chloroplast *trn*L-*trn*F DNA sequences. *New Zealand Journal of Botany*, *45*(1): 67-80.

Simpson, M. G. (2010). *Plant systematics 2nd edition*. Academic press, pp. 465-468.

Smith, S. Y., Stockey, R. A., Nishida, H. & Rothwell, G. W. (2006a). Trawetsia princetonensis gen. et. sp.nov. (Blechnaceae): a permineralized fern from the middle Eocene Princeton chert. *International journal of plant science*, *167*(3): 711-719.

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

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*(5): 1105-1109.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution*, *28*(10): 2731-2739.

Tryon, A. F. (1960). Observations on the juvenile leaves of *Pellaea andromedifolia*. *Contribution from the Gray Herbarium* 187:61–68.

Tryon, R. M. & Tryon, A. F. (1982). *Ferns and allied plants with special reference to tropical America*. New York, NY: Springer-Verlag.

Varigerow, S., Teerkorn, T., & Knoop, V. (1999). Phylogenetic information in the mitochondrial *nad5* gene of pteridophytes: RNA editing and intron sequences. *Plant Biology*, *1*(2): 235-243.

Vijayan, K., & Tsou, C. H. (2010). DNA barcoding in plants: taxonomy in a new perspective. *Current Science (Bangalore)*, *99*(11): 1530-1541.

Xin, Y.M., Cai, X.X., Chang, L., Jing, Y.S., Hui, Y., Kun, L., Ying, J.Z., Ting, G., Xiao, H.P., Jun, Q., & Shi, L. C. (2010). Species identification of medicinal pteridophytes by a DNA barcode marker, the chloroplast *psbA-trnH* intergenic region. *Biology Pharmaceutical Bulletin 33*(199):1919-1924.

Wolf, P. (1997). Evaluation of atpB nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. *American Journal of Botany, 84*(10), 1429-1429.

Wolfe, A.D & dePamphilis, C.W. (1998). The effect of relaxed functional constraints on the photosynthetic gene *rbcL* in photosynthetic and non photosynthetic parasitic plants. *Molecular Biology Evolution, 15(10)*: 1243-1258.

Yatskievych, G. (2003). *Pteridophytes (Ferns)*. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net. Doi: 10.1038/npg.els.0003679.