

RESEARCH NOTE

**A PRELIMINARY MOLECULAR PHYLOGENY OF  
SELAGINELLACEAE OF PENINSULAR MALAYSIA BASED  
ON *rbcL* AND *atpB* MARKERS**

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The order of Spikemosses, or Selaginellales, is represented by a single family, the Selaginellaceae, constituting a single genus, *Selaginella*. Comprising about 750 living species, this family however, does not form a prominent part of the world's vegetation. Selaginellaceae is easily recognized by their dichotomously branching stems that bear ranks of minute leaves in two different sizes (Jermy 1990). The branching systems of Selaginellaceae which can either be pseudopinnate or flabellate are important characters for identification and classification of the species in the genus (Wong 2010). Selaginellaceae is a cosmopolitan family with great diversity and its primarily found in the tropical rainforest (Korall *et al.*, 1999).

Previous systematic studies had reported division of the Selaginellaceae family into numerous groups such as *Selaginella* Pal. Beauv., *Ericetorum* Jermy, *Tetragonostachys* Jermy, *Stachygynandrum* (Pal. Beauv.) Baker and *Heterostachys* Baker (Spring, 1850; Baker, 1883; Hieronymus, 1901; Walton and Alston, 1938; Jermy, 1986; Wong, 1983 & 2010). In Malay Peninsula, the genus *Selaginella* is represented entirely by the species belonging to subgenus *Heterophyllum* Hieron. Subgenus *Heterophyllum* Hieron. characterised by dissimilar-sized leaf, contains a larger number of species with predominant tropical distribution, in comparison to subgenus *Selaginella* Baker, characterised by similar-sized leaf, has a smaller number of species which are mainly found in temperate regions.

In Malaysia, the classification of this family was performed based on morphological characters by Wong (1983 and 2010). Present study aimed to construct a phylogenetic tree to observe the relationship between species of Selaginellaceae in Peninsular Malaysia using molecular data from

chloroplast genome (*rbcL* and *atpB*). Wong (2010) classified genus *Selaginella* to subgenus *Heterophyllum* Hieron., with the following description; leaves in four ranks along the branch axes and distribution in mainly tropical region and the only group encountered in Peninsular Malaysia. The latter subgenus in Peninsular Malaysia appears to be naturally divided as Section *Homostacheae* (with monomorphic sporophylls and cylindrical-quadrangular strobili) and Section *Heterostachyeae* (with dimorphic sporophylls and bilaterally symmetrical strobili) in the system of Ridley (1919), who gave the first account of *Selaginella* in the Malay Peninsula.

Taxonomy and nomenclature of the species were according to Wong (1983 and 2010). A total of 16 taxa were used in this study; *Selaginella argentea*, *S. frondosa*, *S. plana*, *S. ornata*, *S. roxburghii* var. *roxburghii*, *S. intermedia* var. *intermedia*, *S. intermedia* var. *dolichocentrus*, *S. mayeri*, *S. alutacia*, *S. minutifolia*, *S. morganii*, *S. pubescens*, *S. repanda*, *S. willdenowii*, *S. stipulata* and *S. wallichii* (see Table 1). *Huperzia lucidula* (Lycopodiaceae) and *Isoetes laosiensis* (Isoetaceae) were chosen as outgroup. The sister-group relationship between Selaginellaceae, Lycopodiaceae and Isoetaceae has been confirmed in several studies by morphological data (Kenrick and Crane, 1997) and molecular data (Kranz and Huss, 1996; Wikstrom and Kenrick, 1997; Korall *et al.*, 1999).

Total genomic DNA was extracted from silica-dried or fresh fronds using modified CTAB procedure (Doyle and Doyle, 1987). Primers *rbcL* of Korall *et al.* (1999) and “*atpB49*”, “*atpB1592*” of Wolf (1997) were used for PCR amplification from genomic DNA and for sequencing. Twenty-five µl PCR reactions contain 0.5 µl Taq DNA polymerase, 5 X buffer, 25 mM MgCl<sub>2</sub>, 2.0 mM dNTP, and 0.2 mM DNA templates. Reactions were incubated at 94°C for 5 min, then subjected to PCR cycle for 35

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**Table 1.** The specimens collected for this study and their specific location is listed below

SPESES	COLLECTORS & COLLECTION NO.	LOCALITY
<i>Selaginella alutacia</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-09 (UKMB)	Selangor, Ulu Gombak
	Razali Jaman, Masnoryante & Hazwani, NHA2011-11, NHA2011-13 (UKMB).	Selangor, Sg. Semungkis
<i>Selaginella argentea</i>	Razali Jaman & Hazwani, NHA2011-29 (UKMB).	Selangor, Hutan Simpan Kekal UKM
<i>Selaginella frondosa</i>	Razali Jaman & Hazwani, NHA2011-30 (UKMB).	Selangor, Hutan Simpan Kekal UKM
<i>Selaginella intermedia</i> var. <i>dolichocentrus</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-20, NHA2011-21 (UKMB)	Selangor, Fraser's Hill, Pinetree Trail
<i>Selaginella intermedia</i> var. <i>intermedia</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-23 (UKMB).	Pahang, Cameron Highlands, Hutan Lipur Parit Fall
<i>Selaginella mayeri</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-03, NHA2011-04, NHA2011-05, NHA2011-06 (UKMB)	Selangor, Ulu Gombak
	Razali Jaman, Masnoryante & Hazwani, NHA2011-14 (UKMB).	Selangor, Sg. Semungkis
<i>Selaginella minutifolia</i>	Masnoryante & Nurfarahain Zainal, NAZ2012-08 (UKMB)	Selangor, Fraser's Hill, Pinetree Trail
<i>Selaginella morganii</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-24 (UKMB).	Pahang, Cameron Highlands, Hutan Lipur Parit Fall
<i>Selaginella ornata</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-18, NHA2011-19 (UKMB)	Selangor, Fraser's Hill, Jeriau
	Razali Jaman, Masnoryante & Hazwani, NHA2011-22 (UKMB)	Pahang, Cameron Highlands, Hutan Lipur Parit Fall
<i>Selaginella plana</i>	Razali Jaman & Hazwani, NHA2011-31 (UKMB).	Selangor, Hutan Simpan Kekal UKM
<i>Selaginella pubescens</i>	Haja Maideen, Masnoryante & Nurfarahain Zainal, NAZ2013-82 (UKMB)	Pulau Langgun, Langkawi
<i>Selaginella repanda</i>	Haja Maideen, Masnoryante & Nurfarahain Zainal, NAZ2013-82 (UKMB)	Pulau Langgun, Langkawi
<i>Selaginella roxburghii</i> var. <i>roxburghii</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-08 (UKMB)	Selangor, Ulu Gombak
	Razali Jaman, Masnoryante & Hazwani, NHA2011-12 (UKMB)	Selangor, Sg. Semungkis,
	Razali Jaman, Masnoryante & Hazwani, NHA2011-15, NHA2011-17 (UKMB).	Selangor, Ulu Kemensah
<i>Selaginella stipulata</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-16 (UKMB).	Selangor, Ulu Kemensah
<i>Selaginella wallichii</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-01 (UKMB).	Selangor, Ulu Gombak
<i>Selaginella willdenowii</i>	Razali Jaman, Masnoryante & Hazwani, NHA2011-02 (UKMB)	Selangor, Ulu Gombak

times (94°C for 1 min, 40-60°C for 30 sec, and 72°C for 2 min), followed by a final extension of 10 min at 72°C. Double stranded products were purified using Purification Kit for PCR (Vivantis, Malaysia). Purified products were sent to external company, First Base, for sequencing.

DNA sequences were edited and aligned using BioEdit and CLUSTAL W (Thompson *et al.*, 1994). Maximum Parsimony (MP) analysis was performed with PAUP 4.0b10 (Swofford, 2002) using the heuristic search option with 100 random replications. Bootstrap analysis (Felsenstein, 1985) was performed with 1,000 replicates to evaluate internal support.

The 2891 nucleotide segment of the combine data *rbcl* and *atpB* genes revealed no insertions and deletions. Among the sampled species, there were 353 variable sites (12.2%), of which 533 (18.4%) were phylogenetically informative. The length of the existence trees are 1244 steps, a consistency index (CI) of 0.7934, and a retention index (RI) of 0.8085.

Phylogenetic analysis of combined dataset including indel-coded information was formed under parsimony inference. The results showed all species of Selaginellaceae studied were divided into four groups which are Group A, Group B, Group C and

Group D with highly supported bootstrap values ranging at >90%. Group A includes *S. roxburghii* var. *roxburghii*, *S. intermedia* var. *intermedia* and *S. intermedia* var. *dolichocentrus* while Group B included *S. ornata*, *S. pubescens*, and *S. repanda*. Group C divided to two subgroups (namely C1 and C2 with highly supported (bootstrap values = 100%). The first group (C1) contains *S. minutifolia*, *S. morganii* and *S. alutacia* which have close relationships of bilateral strobili. The second subgroup (C2) contains *S. stipulata* and *S. wallichii*, *S. frondosa*, *S. plana*, *S. willdenowii* and *S. mayeri* as a basal group for group C. The last group is Group D with *S. argentea* with high bootstrap values = 100%.

Results are consistent with monophyly of Selaginellaceae (Fig. 1), supported by the previous studied by Korall and Kenrick (2002). Despite its large size (700 species), only a handful of taxonomic groups are widely recognized within Selaginellaceae. Monophyly of the topology has been supported previously by three molecular studies that sampled some of the species of the group based on *rbcl* region e.g. Therrien *et al.* (1999), Therrien and Haufler (2000) and Korall and Kenrick (2002).

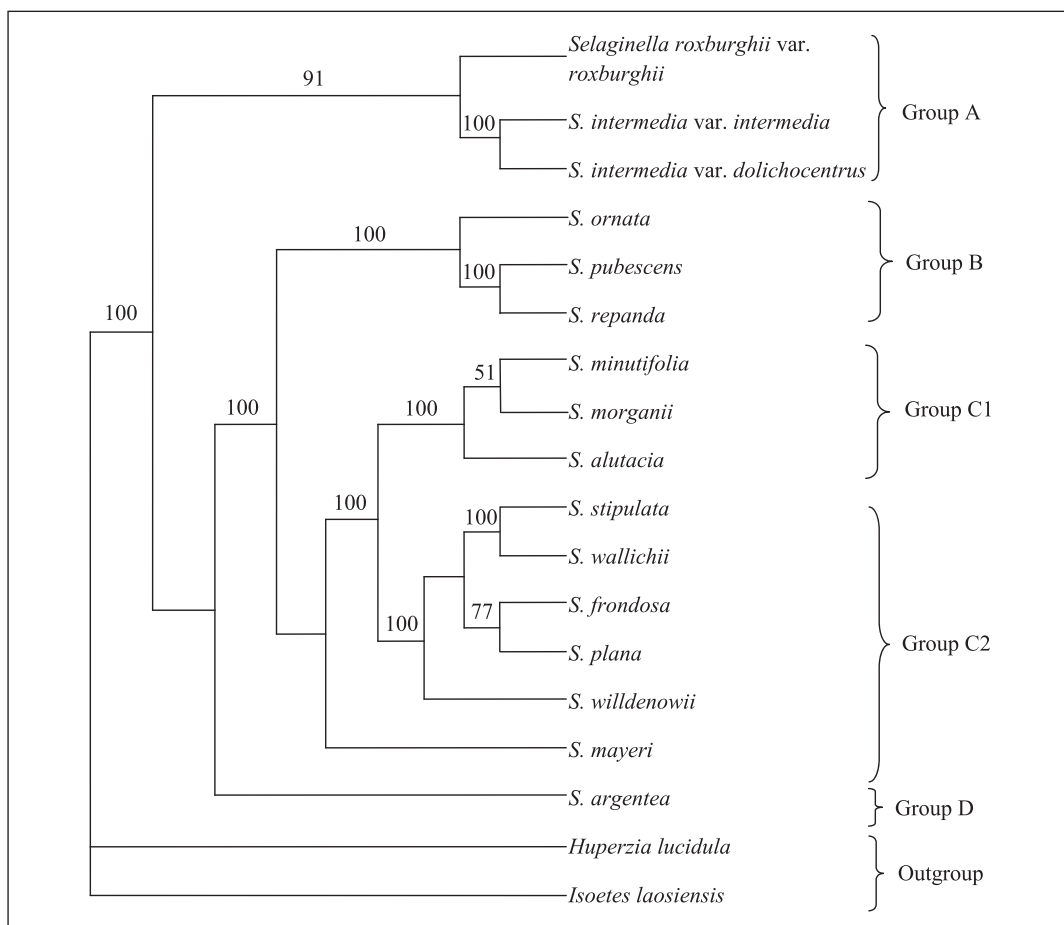


Fig. 1. Strict consensus of most parsimonious trees. Values above branches indicate bootstrap values > 50%.

Based on the results from combined data of *rbcL* and *atpB*, group A contains both pseudopinnate arrays with smaller axes obviously dichotomized and tetragonous type of strobilus. Group B contains species that occur at higher altitudes. Group C1 comprises the species with bilaterally symmetrical strobili and a very small size of plants compared to other species of Selaginellaceae while group C2 comprises those species with a fan-shaped or flabellate array, in which no apparent unidirectional main branch axis is recognizable. The last group with single species *S. argentea* that has ciliolate to ciliate with basally distended upper margin and median leaves with arista often 1/3 to 1/2 lamina length.

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#### REFERENCES

- Baker, J.G. 1883. A synopsis of the genus *Selaginella*. *London Journal of Botany* **21**: 1-5.
- Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11-15.
- Felsenstein, J. 1985. Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal Molecular Evolution* **17**: 368-376.
- Hieronymus, G. 1901. Selaginellaceae. In: Eagler, A. & Prantl, K. (editors). *Die naturlichen pflanzenfamilien*. Vol.1. Ed. Ke-4. Leipzig: Wilhelm Engelmann.
- Jermy, A.C. 1986. Subgeneric names in *Selaginella*. *Fern Gazette* **13**: 117-118.
- Jermy, A.C. 1990. Selaginellaceae. In: Kramer, K.U. & Green, P.S. (editor), *The families and genera of vascular plants*, pg. 39-45. New York: Springer-Verlag.
- Kenrick, P. & Crane, P.R. 1997. *The origin and early diversification of land plants: A cladistic study*. Washington: Smithsonian Institution Press.
- Korall, P., Kenrick, P. & Therrien, J.P. 1999. Phylogeny of Selaginellaceae: evolution of generic/subgeneric relationships based on *rbcL* gene sequences. *International Journal of Plant Sciences* **160**: 585-594.
- Korall, P. & Kenrick, P. 2002. The phylogenetic history of Selaginellaceae based on *rbcL* sequences. *American Journal of Botany* **89**(3): 506-517.
- Kranz, H.D. & Huss, V.A.R. 1996. Molecular evolution pteridophytes and their relationships to seed plants: evidence from complete 18S rRNA gene sequences. *Plant Systematic Evolution* **202**: 1-11.
- Ridley, H.N. 1919. The fern-allies and characeae of the Malay Peninsula. *Journal of the Straits Branch of the Royal Asiatic Society* **80**: 139-161.
- Spring, A.F. 1850. Monographie de la famille des Lycopodiacees. Series 2. *Memoirs of the Royal Belgium Academy of Science* **24**: 1-358.
- Swofford, D.L. 2002. *Phylogenetic analysis using parsimony and other methods, 4. version*. Sunderland: Sinauer Associates, Inc.
- Therrien, J.P. & Haufler, C.H. 2000. Phylogeny and biogeography of *Selaginella* subg. *Tetragonostachys* based on nuclear ribosomal ITS sequence data. *American Journal of Botany* **87**: 98.
- Therrien, J.P., Haufler, C.H. & Korall, P. 1999. *Phylogeny and biogeography of Selaginella* subg. *Tetragonostachys*. In XVI International Botanical Congress, abstracts. pg. 120. USA: St Louis.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jaeanmougin, F. & Hinggens, D.G. 1994. The CLUSTAL W Windows: flexible strategies for multiple sequencing alignments aided by quality analysis tools. *Nucleic Acid Research* **25**: 4875-4882.
- Walton, J. & Alston, A.H.G. 1938. Lycopodiinae. In: Verdoorn, F. (editor). *Manual of Pteridology* page 17. The Hague: Nijhoff.
- Wikstrom, N. & Kenrick, P. 1997. Phylogeny of Lycopodiaceae (Lycopsida) and the relationships of *Phylloglossum drummondii* Kunze based on *rbcL* sequences. *International Journal of Plant Science* **158**: 862-871.
- Wolf, P.G. 1997. Evaluation of *atpB* nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. *American Journal of Botany* **84**(10): 1429 (Abstract).
- Wong, K.M. 1983. Critical observation on Peninsular Malaysia *Selaginella*. *Garden's Bulletin Singapore* **35**(2): 107-135.
- Wong, K.M. 2010. Selaginellaceae. In: Parris, B.S., Kiew, R., Chung, R.C.K., Saw, L.G. & Soepadmo, E. (editors). *Flora of Peninsular Malaysia, Series I: Ferns and Lycophytes* Vol. 1: 49-86. Kuala Lumpur: Forest Research Institute Malaysia.