Blumea 54, 2009: 203–206
www.ingentaconnect.com/content/nhn/blumea

RESEARCH ARTICLE

doi:10.3767/000651909X476166

Davalliaceae in Peninsular Malaysia, a preliminary study based on *trnL-F* region

H. Maideen[1], R.I. Milne[2], M. Gibby[3]

Key words

Davalliaceae
molecular phylogeny
Peninsular Malaysia
trnL-F

Abstract A preliminary molecular analysis based on *trnL-F* region is presented for 17 taxa of *Davalliaceae* in Peninsular Malaysia. Maximum parsimony and Bayesian analysis were conducted on the dataset in order to establish a robust phylogenetic relationship between taxa. The results of analysis indicate incongruence with morphological classification. All genera of *Davalliaceae* in the study area are paraphyletic except *Araiostegia* which is represented by only a single species. In addition it partially agrees with recent phylogeny base on *rbcL* data.

Published on 30 October 2009

INTRODUCTION

Davalliaceae is a moderate-sized family of ferns with about 50–60 species in four currently accepted genera (Nooteboom 1992, 1994, 1998, Schneider et al. 2002), which is restricted to the Old World tropics and subtropics. In Peninsular Malaysia this family is represented by 17 species in five genera (Parris & Latiff 1997). However, in a recent revision of the family Nooteboom (1992, 1994, 1998) reduced the number of taxa, and lumped all *Humata* spp. plus *Scyphularia* spp. into *Davallia,* which he divided into two sections: sect. *Davallia* and sect. *Scyphularia*.

The first molecular study by Tsutsumi & Kato (2005) was based on five continuous chloroplast regions (*atpB, rbcL, accd. atpB-rbcL* spacer, and *rbcl-accd* spacer), and indicated that none of the genera in the family was monophyletic: *Araiostegia* and *Davallia* were divided into two and three clades respectively, and *Humata* and *Scyphularia* were paraphyletic. This finding did not support either the traditional classification of the genus or the division into two sections suggested by Nooteboom (1992, 1994). The present study uses a fast-evolving chloroplast region, *trnL-F,* to infer the phylogeny of *Davalliaceae* in Peninsular Malaysia and to test the generic and sectional classification within the group. The suitability of the *trnL-F* region has already been tested in other studies on fern phylogeny (Schneider et al. 2004a, b, Skog et al. 2004). The present study also has a more complete taxon sampling of Malaysian members of the family than has been used before.

MATERIAL AND METHODS

Ingroup sampling

All species of *Davalliaceae* reported to occur in Peninsular Malaysia by the most recent studies (Parris & Latiff 1997, Nooteboom 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 (University of Tokyo, Japan) and from herbarium specimens (see Table 1 for details of specimens).

Outgroup sampling

Previous studies of fern phylogeny (Tsutsumi & Kato 2005, 2006) have reported the genus *Oleandra* as a sister clade to davallioid ferns. However, *Leucostegia* was also classified as an outgroup because this genus was originally placed within *Davalliaceae* but has since been indicated by molecular data to fall outside it (Tsutsumi & Kato 2006). In the present study, the outgroup consisted of three species, i.e., one representative of *Oleandra* and two of *Leucostegia.*

DNA extraction, sequencing and alignment

Procedures for extraction, amplification and sequencing in this study followed RBGE molecular lab protocols (Clark & Hollingsworth 2006). The primers used are those published previously for *trnL-F* region (Taberlet et al. 1991, Trewick et al. 2002). The sequence was aligned manually using MacClade v4.0 (Maddison & Maddison 2003). Ambiguous regions at the ends of the sequences were excluded. Fifteen gaps were coded using simple indel coding and the multistate gap region method (Simmons & Ochoterena 2000, Simmons et al. 2001).

Phylogenetic analysis

For the phylogenetic reconstruction two different types of analyses were performed: Maximum Parsimony (MP) and Bayesian Analysis (BA). We used PAUP v4.0b10 (Swofford 2002) for the reconstruction using Maximum Parsimony.

All characters were treated as unweighted and unordered. Multistate characters were interpreted as uncertain and gaps were treated as missing.

Heuristic searches were performed for all analyses with 10 000 RANDOM addition sequence replicates using TBR with MULTREES on, STEEPEST DESCENT off, ACCTRAN-optimization and branches collapsed if minimum branch length is zero. Descriptive tree statistics were given by the consistency index (CI), retention index (RI) and rescaled consistency index (RC).

Branch support analyses were carried out using Bootstrap (Felsenstein 1985) and Decay Indices (Bremer 1988). Bootstrap values were calculated using 10 000 replicates with the same

[1] Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia.
[2] Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh, United Kingdom.
[3] Royal Botanic Garden of Edinburgh, United Kingdom.

Table 1 List of species with locality, collection and RBGE accession numbers.

Species	Locality	Collector, collectors' no.	RBGE accession no.
Araiostegia hymenophylloides	Malaysia, Perak, Bukit Larut track to post office	H. Maideen & R. Jaman, HM6035	20051615
Davallia corniculata	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	H. Maideen & R. Jaman, HM6051	20051614b
Davallia denticulata	Malaysia, Negeri Sembilan, Pedas, Ulu Sepri, palm oil estate	H. Maideen & R. Jaman, HM6016a	
	Malaysia, Kedah, Gunung Jerai, summit	H. Maideen & R. Jaman, HM6028	
	Selangor, Bangi, University Campus	H. Maideen & R. Jaman, HM6016b	
Davallia dimorpha	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	H. Maideen & R. Jaman, HM6045	
Davallia divaricata	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	H. Maideen & R. Jaman, HM6005	20051627
	Malaysia, Selangor, Fraser Hills, near Gap (chinese temple)	H. Maideen & R. Jaman, HM6013	
	Malaysia, Perak, Bukit Larut near post office	H. Maideen & R. Jaman, HM6031	20051631
	Malaysia, Perak, Bukit Larut, near post office	H. Maideen & R. Jaman, HM6037	20051642
	Malaysia, Perak, Bukit Larut, near post office	H. Maideen & R. Jaman, HM6042	
	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	H. Maideen & R. Jaman, HM6012	
	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	H. Maideen & R. Jaman, HM6014	
Davallia solida	Malaysia, Kedah, Gunung Jerai, summit	H. Maideen & R. Jaman, HM6017	
	Malaysia, Penang, Penang Hills	H. Maideen & R. Jaman, HM6030	20051641
	Malaysia, Kedah, Gunung Jerai, summit	H. Maideen & R. Jaman, HM6027	
Davallia trichomanoides var. *lorrainii*	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	H. Maideen & R. Jaman, HM6053	
	Malaysia, Kedah, Gunung Jerai, summit	H. Maideen & R. Jaman, HM6018	20051640
	Malaysia, Perak, Gunung Hijau, track to summit	H. Maideen & R. Jaman, HM6041	20051613
Humata angustata	Malaysia, Kedah, Gunung Jerai, summit	H. Maideen & R. Jaman, HM6020	20051632/20051633
	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	H. Maideen & R. Jaman, HM6006	
Humata heterophylla	Malaysia, Kedah, Gunung Jerai, summit	H. Maideen & R. Jaman, HM6024	
Humata parvula	Cult. in Youji Kitaoka's private garden, Ichihara, origin unknown	Chie Tsutsumi, CT1048	
Humata pectinata	Malaysia, Kedah, Gunung Jerai, Tangga Kenari	H. Maideen & R. Jaman, HM6022	20051624/20051635
Humata repens	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	H. Maideen & R. Jaman, HM6002	
	Malaysia, Pahang, Fraser Hills, track to Jeriau waterfall	H. Maideen & R. Jaman, HM6007	20051634
	Malaysia, Kedah, Gunung Jerai, summit	H. Maideen & R. Jaman, HM6019	20051646
	Malaysia, Kedah, Gunung Jerai, Tangga Kenari	H. Maideen & R. Jaman, HM6025	20051634
	Malaysia, Perak, Bukit Larut near post office	H. Maideen & R. Jaman, HM6033	20051645
Humata vestita	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	H. Maideen & R. Jaman, HM6039	20051616
Leucostegia immersa	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	H. Maideen & R. Jaman, HM6040	20051617
Leucostegia pallida	Cult. in Youji Kitaoka's private garden, Ichihara, origin unknown	Chie Tsutsumi, CT1057	
Scyphularia triphylla	Malaysia, Johore, Gunung Pulai	H. Maideen & R. Jaman, HM6046	20051619/20051622

settings as above, except with only 1 RANDOM addition per replicate. Decay Index was calculated with default settings in AutoDecay v4.0 (Eriksson 1999).

Bayesian analysis

Parameters and the evolutionary model for the region was selected with the assistance of Modeltest v3.07 (Posada & Crandall 1998, 2001, Posada & Buckley 2004). The parameter and model based on the Akaike Information Criterion (AIC) was used. 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.

RESULTS

Sequence alignment and model selection

The aligned *trnL-F* sequence data matrix contains 924 characters, of which 636 are parsimony informative. The AIC selected model for *trnL-F* was K81 uf+1 (two transversion-parameter model I unequal frequencies; Kimura (1981)).

Maximum parsimony

In the maximum parsimony (MP) analysis, the analysis including gap characters produced a single most parsimonious tree of 447 steps with CI = 0.88, RI = 0.94 and RC = 0.82. These values are relatively high indicating that the number of homoplastic characters is low. The bootstrap majority tree was less highly resolved, with a major polytomy near the base of the cladogram. Fig. 1 presents the singel most parsimonious tree. The topology in Fig. 1 shows that the ingroup consists of two lineages. The first lineage is formed by *Araiostegia hymenophylloides*, the second one is weakly supported (bs = 55 %, d = 1) and consists of a trichotomy of two partially resolved groups, a very weakly supported clade (DCII): including S*cyphularia*–*D. solida*, a well-supported clade consisting of all representatives of *D. trichomanoides* and a well-supported clade consisting of a Davallia clade (DC1) and a Humata clade (HC).

Davallia clade I has *D. denticulata* as sister to *D. dimorpha* and *D. divaricata* (bs = 99 %, d = 7) and the Humata clade consists of all *Humata* spp. plus *D. corniculata* (bs = 99 %, d = 1). *Humata heterophylla* is a sister to *D. corniculata* and other *Humata* species with high support (bs = 99 %, d = 10).

Bayesian analysis

The topology of the Bayesian majority rule tree is identical to the MP analysis, except that *Scyphularia triphylla* is sister to the Davallia clade with a low posterior probability value (pp = 0.76). The posterior probability for the nodes in the phylogeny ranges between 0.59 for the node joining the basal trichotomy to 1.00.

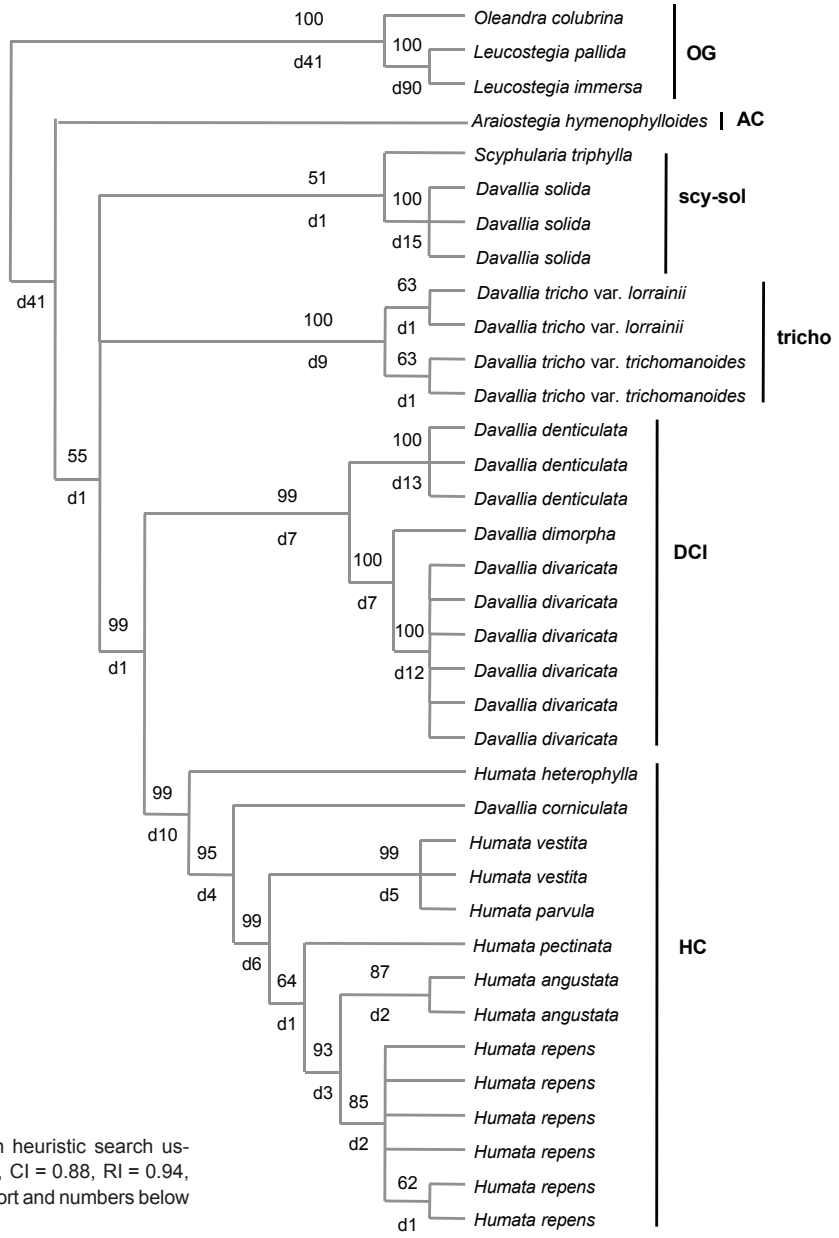

Fig. 1 Phylogenetic tree based on *trnL-F* sequences with heuristic search using maximum parsimony analysis. Tree length = 447 steps, CI = 0.88, RI = 0.94, RC = 0.82. Numbers above branches indicate bootstrap support and numbers below branches indicate decay indices.

DISCUSSION AND CONCLUSION

The monophyly of Davalliaceae

The phylogenetic relationships indicated by our analysis of the *trnL-F* region are 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 others (Tsutsumi & Kato 2005, 2006, Tsutsumi et al. 2008). *Davalliaceae* (excluding *Leucostegia*) was shown by our data to comprise five major lineage or groups, namely the *Araiostegia hymenophylloides* (AC), *D. solida*, *D. trichomanoides*, a Davallia clade 1 (DC1) and a Humata clade (HC). The position of *Scyphularia triphylla* could not be established with any confidence.

The partitioning of clades/groups is almost the same as in Tsutsumi & Kato (2005), although the sister relationship of *Araiostegia* to all other clades or groups, is only weakly supported with bootstrap value 55 %, decay value 1 and posterior probability 59 %. In both analyses (MP and BA), *D. solida* and *D. trichomanoides* form a polytomy with other clades. In the Humata clade, *Humata heterophylla* is sister to all other *Humata* species, including *D. corniculata,* with high support (bs = 99 %, d = 10). As no study has been done on the same gene in other members of *Davalliaceae*, a further comparison with other data could not be made.

The present study has confirmed that *Scyphularia triphylla* is nested within Davallia, and should therefore be reduced to Davallia, but as only one *Scyphularia* species was examined the monophyly of this genus could not be tested. The present study also confirms that neither Davallia nor Humata are monophyletic, and based on this evidence plus that of Tsutsumi & Kato (2005, 2006), these genera need to be merged or redefined. The genus *Humata* could be made monophyletic by including *Davallia corniculata* but *Davallia* would then still not be monophyletic. Before formal taxonomic changes are made, however, it would be valuable to obtain additional data from further DNA regions, in order to confirm beyond doubt that the relationships resolved here reflect biological reality.

Acknowledgements The authors are grateful to all RBGE science staff, the molecular lab staff in the Department of Botany (Natural History Museum, London), herbaria of KEP, KEW, L, NHM, RBGE, SING and UKMB. The first author also like to thank R. Jaman (UKMB) for support in field work, C. Tsutsumi (University of Tokyo) for DNA material, M. Moller (RBGE) and H. Schneider (NHM) for the molecular analysis and valuable comments. This research was funded by Universiti Kebangsaan Malaysia and Malaysian government.

REFERENCES

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

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

Eriksson T. 1999. AutoDecay v4.0. Bergius Foundation, Royal Swedish Academy of Sciences, Stockholm.

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

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

Maddison WP, Maddison DR. 2003. MacClade, analysis of phylogeny and character evolution. v4.06 OS X. Sinauer, Sunderland, Massachusetts.

Nooteboom HP. 1992. Notes on Davalliaceae I. The genera Araiostegia, Davallodes, Leucostegia and Gymnogrammitis. Blumea 37: 165–187.

Nooteboom HP. 1994. Notes on Davalliaceae II. A revision of the genus Davallia. Blumea 39: 151–214.

Nooteboom HP. 1998. Davalliaceae. Flora Malesiana, Ser. II, Vol. 3: 235–276.

Parris BS, Latiff A. 1997. Towards a pteridophyte flora of Malaysia: A provisional checklist of taxa. Malayan Nature Journal 50: 235–280.

Posada D, Buckley TR. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike Information Criterion and Bayesian approaches over Likelihood Ratio Tests. Systematic Biology 53, 5: 793–808.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 490–500.

Posada D, Crandall KA. 2001. Selecting the best-fit model of nucleotide substitutions. Systematic Biology 50, 4: 580–601.

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

Schneider H, Smith AR, Cranfill R, Haufler CH, Ranker TA, Hildebrand T. 2002. Gymnogrammitis dareiformis is a polygrammoid fern (Polypodiaceae) – Resolving an apparent conflict between morphological and molecular data. Plant Systematics and Evolution 234: 121–136.

Schneider H, Smith AR, Cranfill R, Hilderbrand T, Haufler CH, Ranker TA. 2004b. Unreaveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): exploring aspects of the diversification of epiphytic plants. Molecular Phylogenetics and Evolution 31, 3: 1041–1063.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49, 2: 369–381.

Simmons MP, Ochoterena H, Carr TG. 2001. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. Systematic Biology 50, 3: 454–462.

Skog JE, Mickel JT, Moran RC, Volovsek M, Zimmer EA. 2004. Molecular studies of representative species in the genus Elaphoglossum (Dryopteridaceae) based on cpDNA sequences rbcL, trnL-F and rps4-trnS. International Journal of Plant Sciences 165: 1063–1075.

Swofford DL. 2002. PAUP: Phylogenetic analysis using parsimony (and other methods), v4.0b10. Sinauer, Sunderland, Massachusetts.

Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primer for amplification of three non coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105–1109.

Trewick SA, Morgan-Richards M, Russell SJ, Henderson S, Rumsey FJ, Pintér I, Barrett JA, Gibby M, Vogel JC. 2002. Polyploidy, phylogeography and Pleistocene refugia of the rockfern Asplenium ceterach: evidence from chloroplast DNA. Molecular Ecology 11: 2003–2012.

Tsutsumi C, Kato M. 2005. Molecular phylogenetic study on Davalliaceae. Fern Gazette 17, 2: 143–158.

Tsutsumi C, Kato M. 2006. Evolution of epiphytes in Davalliaceae and related ferns. Botanical Journal of the Linnean Society 151: 495–510.

Tsutsumi C, Zhang XC, Kato MH. 2008. Molecular phylogeny of Davalliaceae and implications for generic classification. Systematic Botany 33: 44–48.