



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

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Key words

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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.

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INTRODUCTION

Davalliaceae is a moderate-sized family of ferns with about 50–60 species in four currently accepted genera (Nootboom 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 Nootboom (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 Nootboom (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, Nootboom 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 MULTITREES 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

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

Species	Locality	Collector, collectors' no.	RBGE accession no.
<i>Araiostegia hymenophylloides</i>	Malaysia, Perak, Bukit Larut track to post office	H. Maideen & R. Jaman, HM6035	20051615
<i>Davallia corniculata</i>	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	H. Maideen & R. Jaman, HM6051	20051614b
<i>Davallia denticulata</i>	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	
<i>Davallia dimorpha</i>	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	H. Maideen & R. Jaman, HM6045	
<i>Davallia divaricata</i>	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	
<i>Davallia solida</i>	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	
<i>Davallia trichomanoides</i> var. <i>lorrainii</i>	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
<i>Humata angustata</i>	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	
<i>Humata heterophylla</i>	Malaysia, Kedah, Gunung Jerai, summit	H. Maideen & R. Jaman, HM6024	
<i>Humata parvula</i>	Cult. in Youji Kitaoka's private garden, Ichihara, origin unknown	Chie Tsutsumi, CT1048	
<i>Humata pectinata</i>	Malaysia, Kedah, Gunung Jerai, Tangga Kenari	H. Maideen & R. Jaman, HM6022	20051624/20051635
<i>Humata repens</i>	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
<i>Humata vestita</i>	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	H. Maideen & R. Jaman, HM6039	20051616
<i>Leucostegia immersa</i>	Malaysia, Pahang, Cameron Highlands, track to G. Brinchang	H. Maideen & R. Jaman, HM6040	20051617
<i>Leucostegia pallida</i>	Cult. in Youji Kitaoka's private garden, Ichihara, origin unknown	Chie Tsutsumi, CT1057	
<i>Scyphularia triphylla</i>	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 *Scyphularia*–*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.

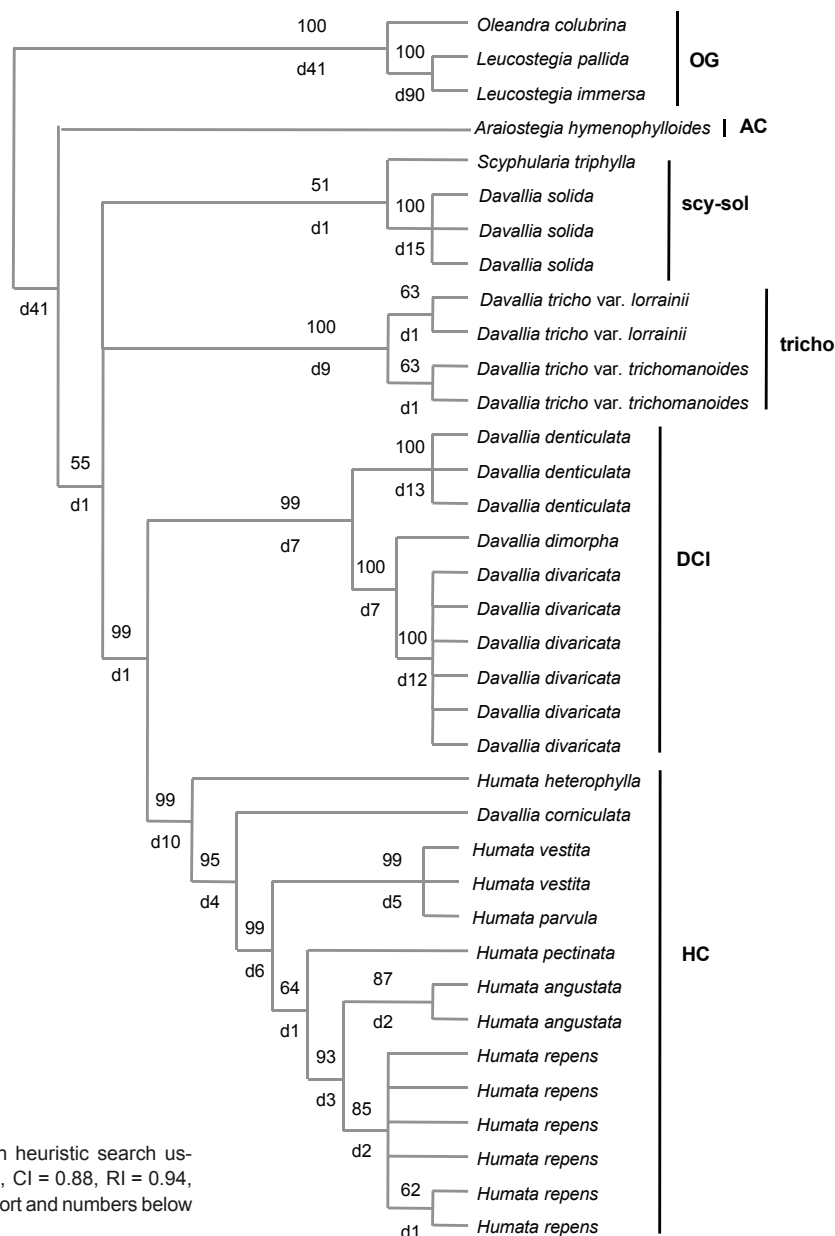


Fig. 1 Phylogenetic tree based on *trnL-F* sequences with heuristic search using maximum parsimony analysis. Tree length = 447 steps, CI = 0.88, RI = 0.94, RC = 0.82. Numbers above branches indicate bootstrap support and numbers below branches indicate decay indices.

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.

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