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Phylogeny of *Davallia* (Davalliaceae) from Sumatra and Mentawai Islands, Indonesia: Evidence from *trnL-F* Intergenic Spacer

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Abstract. *Mildawati, Sobir, Sulistijorini, Chikmawati T. 2023. Phylogeny of* Davallia *(Davalliaceae) from Sumatra and Mentawai Islands, Indonesia: evidence from* trnL-F *Intergenic Spacer. Biodiversitas 24: 4589-4596. Davallia* is a member of the family Davalliaceae, which has a wide distribution in the Malesia region. The classification of this genus based on morphological and molecular data shows differences that cause the relationship among species to be debated until now. This study aimed to examine the phylogeny of *Davallia* from Sumatra and Mentawai Islands, Indonesia based on *trnL-F* intergenic spacer (IGS) sequence data. A total of 26 accessions representing 10 *Davallia* species were selected as ingroup and *Oleandra articulata* (Sw.) C.Presl as an outgroup was downloaded from Gen-Bank (accession number KF667613.1). Data analysis using the Maximum Parsimony (MP) method confirmed *Davallia* into 2 clades with divided into 7 subclades. Results showed that *trnL-F* IGS sequence as a non-coding gene explains the grouping species in *Davallia*. *trnL-F* IGS sequence can estimate species identity caused by changes in nucleotide bases from mutation. The MP analysis showed each species was resolved as monophyletic. Based on the phylogenetic tree, the longest branching was found in *D. corniculata*, suggesting that this species is the most primitive in the genus of *Davallia* in this region. Thus, the *trnL-F* marker effectively showed the relationship among species in *Davallia* in Sumatra and Mentawai Islands.

Keywords: *Davallia*, Mentawai Islands, molecular data, monophyletic, Sumatra, *trnL-F*

INTRODUCTION

Pteridophytes are vascular plants that produce spores and have a unique life cycle in the form of free-living gametophyte and sporophyte generations (Haufler et al. 2016). Pteridophytes vary in size, shape, color, and spore surface pattern (Zhou et al. 2017; Olejnik et al. 2018; Wei et al. 2018). Taxonomic issues in the classification of pteridophytes are not only studied using morphological evidence, but are linked to the study of evolutionary history using a molecular phylogeny approach. Several researchers have consistently studied the molecular data of pteridophytes, such as *Deparia* (Kuo et al. 2016), *Athyrium*, *Anisocampium*, and *Diplazium* (Athyriaceae) (Wei et al. 2018), and *Phlegmariurus* (Lycopodiaceae) (Bauret et al. 2018).

Davallia is a member of the family Davalliaceae and has a rich number of species in the world with 65 accepted species (PPG 2016). They are distributed in tropical and subtropical areas in the world. In Malesia, as many as 23 species of *Davallia* have been recorded, of which 9 species are endemic (Nooteboom 1998). There are 17 species in the Malay Peninsula (Parris and Latiff 1997), and about 13 species are reported in Sumatra and the nearby islands (Mildawati et al. 2022). The genus *Davallia* showed differences in morphological characteristics in the form of peltate or pseudo peltate scales on the rhizome, leaf type shape, and the various number of leaflets (Nooteboom 1998).

Classification by morphological characters shows limitations in describing the relationship among species, because some characteristics still need to be revealed. Therefore, it must be supported by an approach using molecular characters to determine the species' rank in *Davallia*. A molecular study of 29 species of Davalliaceae in China was constructed using 5 plastid DNA that has proven to explain species concept (Ma et al. 2018). Analyzed a molecular phylogeny of 36 species in 5 genera of Davalliaceae shows that no *Davallia* phylogenetic trees are monophyletic (Tsutsumi and Kato 2005), such as phylogenetic studies also represent a paraphyletic grouping in *Davallia* in Peninsular Malaysia (Maideen et al. 2009) and did not match the morphological classification. A paraphyletic grouping in *Davallia* is also supported by differences in the number of genera in this family. Smith et al. (2006) classified Davalliaceae into 4-5 genera, Tsutsumi et al. (2008) proposed 5 genera with 2 sections, Christenhusz et al. (2011) suggested 2 genera, and Xing et al. (2013) revealed 5 genera. Then, Tsutsumi et al. (2016) published only 1 genus of *Davallia* with no infrageneric classifications. The difference in the classification results

raises taxonomic problems related to the position of the *Davallia* species.

This research is a continuation of a research conducted by Mildawati et al. (2022) based on the morphological characters of *Davallia* in Sumatra and the small islands around it. This study uses the molecular marker *trnL-F* IGS in the non-coding cpDNA genome. These markers have been easy to isolate and purify and very conservative, with a low evolutionary rate for the reconstruction of phylogenetic trees for lower taxa in plants (Surya and Hari 2017). The *trnL-F* region which consists of the *trnL* intron and the *trnL-F* spacer has become one of the most widely used chloroplast markers for phylogenetic analyses in plants (Pirie et al. 2007). The molecular markers from cpDNA to resolve species relationships based on evolution are expected to clarify species levels in the *Davallia* in Sumatra and Mentawai Islands which has never been reported. Therefore, this study aimed to analyze and reconstruct the molecular phylogeny of *Davallia* (Davalliaceae) from Sumatra and Mentawai Islands, Indonesia based on *trnL-F* intergenic spacer (IGS) sequence data.

MATERIALS AND METHODS

Plant Materials

Sampling collections of *Davallia* were obtained from several National Parks (NP) in Sumatra (Kerinci Seblat NP, Bukit Tiga Puluh NP, Siberut NP, Gunung Leuser NP, Bukit Barisan Selatan NP, and Bukit Barisan Forest Park) and Mentawai Islands (Sipora Island, North Pagai Island, and South Pagai Island) (Table 1; Figure 1). Mentawai Islands is located about 150 km from the Sumatra mainland, Indonesia. All locations and samples in this study were the same as Mildawati et al. (2022). The Specimens were deposited in Herbarium ANDA, Universitas Andalas, Padang, Indonesia. Plant materials used in the study were carried out on 26 accessions representing 10 species of *Davallia* and *Oleandra articulata* (Sw.) C.Presl as outgroup (http: //www.ncbi.nlm.nih.gov/) (Table 1).

Procedures

DNA isolation

DNA was isolated from 10-15 of dry leaves using Plant DNA Kit (Bioline). The isolation results were electrophoresed using 0.8% agarose gel to analyze DNA qualitatively.

DNA amplification and electrophoresis

DNA amplification was using the molecular marker of *trnL-F* IGS (non-coding DNA) in each of the DNA sample. PCR amplification was carried out by reacting 25 µL of the solution, which consisted of 3 µL DNA template (100 ng/µl), 9 µL ddH_2O, and 1 µL primer *trnL-F* reverse (5'-GGTTCAGTCCCTCTATCCC -3') dan forward (5'-ATTTGAACTGGTGACACGAG-3') respectively (Taberlet et al. 1991), and 11 µL of MyTaq Extract-PCR Kit (Bioline BIO-21126). The PCR mixtures were placed in a GeneAmp PCR System 2400 thermal cycler (Perkin Elmer, USA) and performed to the following PCR setting: initial denaturation at 94°C for 3 minutes; denaturation at 94°C for 30 seconds, annealing at 55°C for 60 seconds, extension at 72°C for 30 seconds (repeated by 35-40 cycles); and final extension at 72°C for 5 minutes (Li et al. 2017). The results of the amplification of *Davallia* and its close relatives with the *trnL-F* IGS. marker were visualized with 0.8% agarose gel electrophoresis, then sequenced.

DNA sequencing

Sequencing was performed using Seqman II (DNA Star Lasergene, Wisconsin, USA), which was sent to the First Base Laboratory, Malaysia.

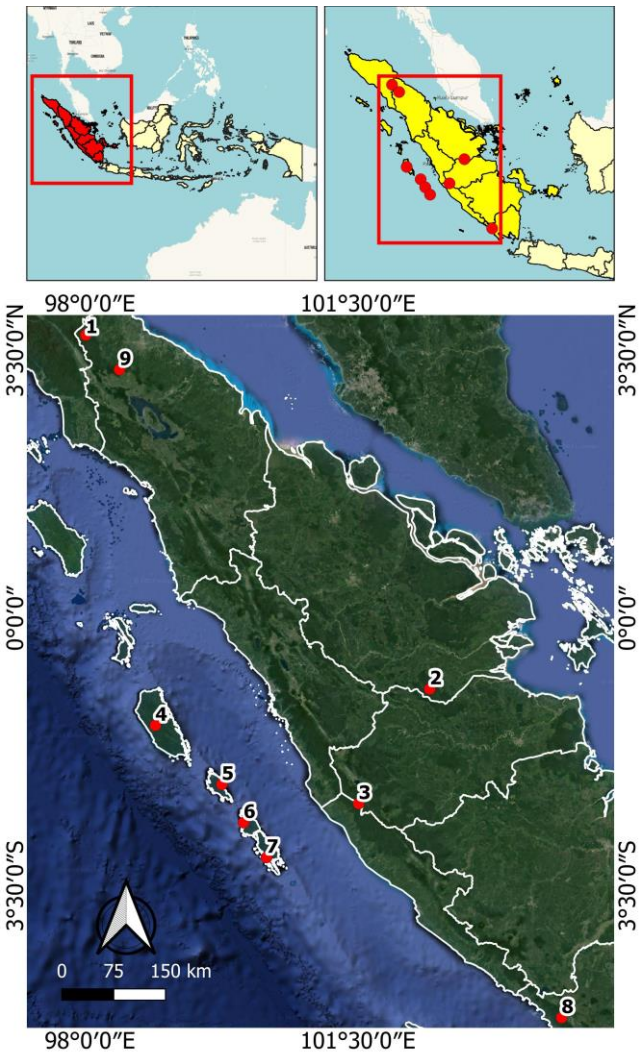

Figure 1. Sampling locations *Davallia* in Sumatra and Mentawai Islands, Indonesia: 1. Gunung Leuser NP, 2. Bukit Tiga Puluh NP, 3. Kerinci Seblat NP, 4. Siberut NP, 5. Sipora Island, 6. North Pagai Island, 7. South Pagai Island (PSS), 8. Bukit Barisan Selatan NP, 9. Bukit Barisan Forest Park

Data analysis

Analysis of DNA sequence with the best quality was carried out to determine the relationship among species. The phylogeny analysis is shown by a phylogenetic tree (cladogram) obtained by alignment using the Clustal W program. Phylogeny analysis used *O. articulata* as an outgroup (KF667613.1) obtained from GenBank-NCBI, because it was the closest sister of *Davallia*. Maximum Parsimony (MP) was applied to evaluate possible tree topologies using MEGA X (Molecular Evolutionary Genetic Analysis) software. Branch support was evaluated through 1000 replicates of bootstrap analysis (Kumar et al. 2018). Principal Component Analysis (PCA) was conducted to assess the grouping of *Davallia* species based on genetic distance data using the free software R (R Core Team 2018).

RESULTS AND DISCUSSION

DNA band profile of *Davallia*

The results of multiple alignments of *trnL-F* IGS sequences of *Davallia* showed a high homology or nucleotide base similarity value (Figure 2). The *trnL-F* IGS nucleotide sequence bases have similarities and differences in all *Davallia* species. DNA band visualization showed that the *trnL-F* IGS sequences were successfully amplified using cpDNA forward and reverse primers. The *trnL* (UAA) 3'exon sequence and the *trnF* gene (GAA) are non-coding regions between the *trnL-F* genes in plant cpDNA usually used as markers for plant identification, such as selected species of Annonaceae (Lestari and Azrianingsih 2019). The *trnL-F* IGS sequence has the highest rate of evolution compared to other sequences, such as *matK* and *rbcL* (Chen et al. 2013). This partial sequence also has a relatively short size (350-600 bp) and is easy to amplify and analyze (Taberlet et al. 1991). The *trnL-F* IGS sequences have been used to reveal the phylogenetic relationships of various groups of ferns, such as the gametophyte Vittarioid (Pteridaceae) (Chen et al. 2013), the genus *Tectaria* (Tectariaceae) (Ding et al. 2014), and the family Hypodematiaceae (Fan et al. 2022).

Variations of *trnL-F* sequence in *Davallia*

The variations of nucleotide base percentages and Gen Bank accession numbers in *Davallia* species originating from Sumatra and Mentawai Islands are presented in Table 2. Results of DNA sequences show that the length of DNA sequences observed ranged from 321 to 553 bp, length mean of 350 bp, aligned length of 321 bp with 66 constant traits (conserve), 67 uninformative traits, and 205 informative parsimony traits. The average nucleotide frequencies in the *trnL-F* IGS sequences were 30.13% (T), 20.57% (C), 30.76% (A), and 18.54% (G) (Table 2). The *trnL-F* IGS sequences have a higher percentage of AT (60.89%) than GC (39.11%), indicating a bias during the mutation process because it was thought that AT-rich genomes arise from AT-biased mutation patterns.

Table 1. *Davallia* from Sumatra and Mentawai Islands, Indonesia were used in the study

Species	Collector no.	Source	GenBank acc. no.
D. corniculata T. Moore	DC 082	Kerinci Seblat NP (Jambi Province)	OQ077741
D. denticulata (Burm.f.) Mett.	DD 016	Kerinci Seblat NP (Jambi Province)	OQ077742
D. denticulata (Burm.f.) Mett.	DD 172	Gunung Leuser NP (Aceh Province)	OQ077743
D. denticulata (Burm.f.) Mett.	DD 97	Bukit Tiga Puluh NP (Riau Province)	OQ077744
D. denticulata (Burm.f.) Mett.	DD 194	Bukit Barisan Selatan NP (Lampung Province)	OQ077745
D. denticulata (Burm.f.) Mett.	DD 114	Sipora Island, West Sumatra Province	OQ077746
D. denticulata (Burm.f.) Mett.	DD 139	North Pagai Island (West Sumatra Province)	OQ077747
D. denticulata (Burm.f.) Mett.	DD 127	South Pagai Island (West Sumatra Province)	OQ077748
D. divaricata Blume	DV 68	Kerinci Seblat NP (Jambi Province)	OQ077749
D. heterophylla Sm.	DH 144	North Pagai Island (West Sumatra Province)	OQ077750
D. hymenophylloides (Blume) Kuhn	DHY 78	Kerinci Seblat NP (Jambi Province)	OQ077751
D. pectinata Sm.	DP 175	Bukit Barisan Forest Park (North Sumatra Province)	OQ077752
D. pectinata Sm.	DPEC 75	Kerinci Seblat NP (Jambi Province)	OQ077753
D. pentaphylla Blume	DPEN 83	Kerinci Seblat NP (Jambi Province)	OQ077754
D. repens (L.f.) Kuhn	DR 18	Kerinci Seblat NP (Jambi Province)	OQ077755
D. repens (L.f.) Kuhn	DR 177	Bukit Barisan Forest Park (North Sumatra Province)	OQ077756
D. solida (G.Forst.) Sw. var. *solida*	DS 148	Gunung Leuser NP (Aceh Province)	OQ077757
D. solida (G.Forst.) Sw. var. *solida*	DS 33	Siberut NP (West Sumatra Province)	OQ077758
D. solida (G.Forst.) Sw. var. *solida*	DS 106	Sipora Island (West Sumatra Province)	OQ077759
D. solida (G.Forst.) Sw. var. *solida*	DS 55	Kerinci Seblat NP (Jambi Province)	OQ077760
D. solida (G.Forst.) Sw. var. *solida*	DS 94	Bukit Tiga Puluh NP (Riau Province)	OQ077761
D. solida (G.Forst.) Sw. var. *solida*	DS 124	South Pagai Island (West Sumatra Province)	OQ077762
D. solida (G.Forst.) Sw. var. *solida*	DS 143	North Pagai Island (West Sumatra Province)	OQ077763
D. solida (G.Forst.) Sw. var. *solida*	DS 195	Bukit Barisan Selatan NP (Lampung Province)	OQ077764
D. trichomanoides Blume	DS 74	Kerinci Seblat NP (Jambi Province)	OQ077765
D. trichomanoides Blume	DT 173	Bukit Barisan Forest Park (North Sumatra Province)	OQ077766
Oleandra articulata (Sw.) C.Presl		GenBank (outgroup)	KF667613.1

Therefore, variations in AT-GC content were probably caused by the combination of genomic substitution patterns being biased toward GC or AT (Reichenberger et al. 2015), which indicates the high repetition and variation of AT bases in the noncoding cpDNA region (Bonatelli et al. 2013). The DNA sequences of various species have a unique composition of nucleotide bases. This study shows that the *Davallia* are included in species with high A and T contents. This result is in accordance with previous studies on *Bouea* (Harsono et al. 2017) and *Baccaurea* (Gunawan 2020), which explained that most of the nucleotide compositions in the noncoding region of cpDNA were A and T bases. The high variation of AT bases in the noncoding region of *trnL-F* IGS will result in mutation rates, so information on base mutations that occur even though they are small is very useful for analyzing phylogenetic study in *Davallia.*

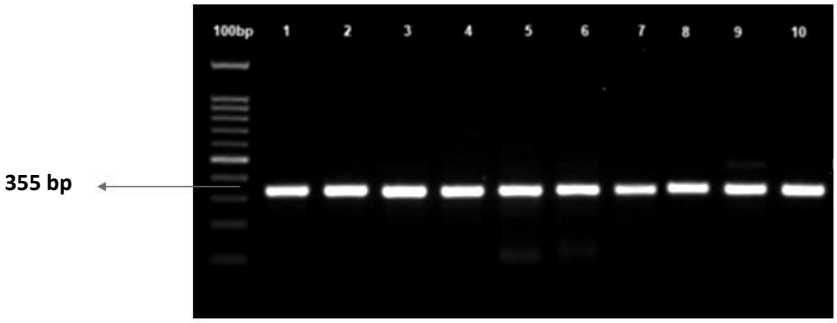

Figure 2. Visualization of PCR products was examined using 0.8% agarose: 1. *D. corniculata* (OQ077741), 2. *D. denticulata* (OQ077742-OQ077748), 3. *D. divaricata* (OQ077749), 4. *D. heterophylla* (OQ077750) 5. *D. hymenophylloides* (OQ077751) 6. *D. pectinata* (OQ077752- OQ077753), 7. *D. pentaphylla* (OQ077754) 8. *D. repens* (OQ077755- OQ077756) 9. *D. solida* var. *solida* (OQ077757- OQ077764) 10. *D. trichomanoides* (OQ077765- OQ077766)

Table 2. The variations AT and GC sequences of *trn*L-F IGS 10 *Davallia* species used in the study

Spesies	GenBank acc. no.	T(U) %	C %	A %	G %	AT %	GC %
Davallia corniculata	OQ077741	34.80	17.57	27.03	20.61	61.82	38.18
D. denticulata	OQ077742	27.03	22.64	30.74	19.59	57.77	42.23
D. denticulata	OQ077743	27.70	22.30	30.74	19.26	58.45	41.55
D. denticulata	OQ077744	27.70	22.30	30.74	19.26	58.45	41.55
D. denticulata	OQ077745	27.70	22.30	30.74	19.26	58.45	41.55
D. denticulata	OQ077746	27.70	22.30	30.74	19.26	58.45	41.55
D. denticulata	OQ077747	27.70	22.30	31.08	18.92	58.78	41.22
D. denticulata	OQ077748	27.70	22.30	31.08	18.92	58.78	41.22
D. divaricata	OQ077749	30.41	20.27	31.76	17.57	62.16	37.84
D. heterophylla	OQ077750	29.39	20.95	30.41	19.26	59.80	40.20
D. hymenophylloides	OQ077751	32.09	16.89	32.43	18.58	64.53	35.47
D. pectinata	OQ077752	33.11	19.26	31.08	16.55	64.19	35.81
D. pectinata	OQ077753	33.78	19.59	30.07	16.55	63.85	36.15
D. pentaphylla	OQ077754	31.76	21.62	28.04	18.58	59.80	40.20
D. repens	OQ077755	30.41	19.26	33.11	17.23	63.51	36.49
D. repens	OQ077756	30.41	19.26	32.43	17.91	62.84	37.16
D. solida var. *soldida*	OQ077757	29.73	20.27	31.42	18.58	61.15	38.85
D. solida var. *soldida*	OQ077758	29.73	20.27	31.08	18.92	60.81	39.19
D. solida var. *soldida*	OQ077759	30.07	18.92	32.09	18.92	62.16	37.84
D. solida var. *soldida*	OQ077760	29.73	20.27	31.08	18.92	60.81	39.19
D. solida var. *soldida*	OQ077761	30.07	20.27	32.43	17.23	62.50	37.50
D. solida var. *soldida*	OQ077762	29.73	21.28	30.41	18.58	60.14	39.86
D. solida var. *soldida*	OQ077763	29.73	21.28	30.41	18.58	60.14	39.86
D. solida var. *soldida*	OQ077764	29.73	20.95	30.41	18.92	60.14	39.86
D. trichomanoides	OQ077765	30.41	21.96	27.70	19.93	58.11	41.89
D. trichomanoides	OQ077766	30.74	21.62	30.74	16.89	61.49	38.51
Oleandra articulata	KF667613.1	34.29	17.46	30.48	17.78	64.76	35.24
Average		30.13	20.57	30.76	18.54	60.89	39.11

Phylogenetic analysis of *Davallia* from Sumatra and Mentawai Islands

A phylogenetic tree among species of *Davallia* in Sumatra and Mentawai Islands is shown in Figure 3. The topology of the phylogenetic tree was constructed based on MP analysis. The phylogenetic tree is monophyletic, which means that the group has one ancestor that inherits genetic, morphological, and biochemical traits in all its descendants. Member of this group is very closely related to one another with bootstrap value of 100%, it means support the phylogenetic tree with the class of bootstrapping values are excessive (>85%) (Vanderlaan et al. 2013). The phylogenetic tree forms branches with a total length of 5.43. This indicates that even though a species is grouped in one branch, the mutation rate is different due to differences in the influence of genetic and environmental factors. Genetic factors include the diversity of nitrogenous bases found in intraspecies and interspecies, while environmental factors can be studied through the geological history of the Sumatra and Mentawai Islands. Based on geological factors, Sumatra and Mentawai Islands have been separated since 500,000 years ago (Sargis et al. 2014).

The phylogenetic tree formed 2 ingroup clades and 1 outgroup. The ingroup group is divided into 2 major clades, namely clades I and II. Clade II only consisted of *D. corniculata* with a bootstrap value of 78%. This clade showed the longest branching compared to the other branches and was closest to the outgroup, so it was assumed this species was the most primitive of the genera *Davallia* analyzed. Clade I consisted of another species analyzed with an average bootstrap value of 80%. Clade I consists of 7 subclades with various bootstrap values. The bootstrap value can be used to assess the stability of the phylogenetic tree branches formed. If the bootstrap value is above 95%, the branch formed is stable; otherwise, if the bootstrap value is below 70%, the branching formed is unstable (Huang et al. 2021). All *Davallia* species form relatively stable branches, because they have an average bootstrap value above 95%. The lowest bootstrap value is only found in the first clade, which separates main Clade I and Clade II (*D. corniculata* and other *Davallia* species) while the highest bootstrapping value is found in clade 7 with bootstrap value of 100% in Clade I (*D. pentaphylla*, *D. pectinata*, and *D. hymenophylloides*, *D. trichomanoides*, *D. repens*, *D. divaricata*, and *D. heterophylla*).

Based on MP analysis, the phylogenetic tree produced Consistency Index (CI) of 0.69, Retention Index (RI) of 0.71, and Homoplasy Index (HI) of 0.31. The phylogenetic tree formed has a branching consistency index value above 0.50 and a homoplasy index below 0.5, so this tree is categorized as strong enough (Ding et al. 2014). In biosystematics, the acceptable phylogenetic tree topology is monophyletic and dichotomous, has a high bootstrap value, and the clades formed are consistent and solid. In monophyletic groupings, all of their descendants come from the same ancestor, as has also been found in the *Humata vestita* and *H. parvula* groupings (Tsutsumi et al. 2008).

The relationship analysis among species in the genus *Davallia* was also carried out using Principal Analysis Components (PCA). This analysis uses molecular data in the form of genetic distance from 26 accessions of *Davallia* in Sumatra and Mentawai Islands and 1 outgroup (Figure 4). The results showed all *Davallia* were divided into 3 main clades (including 7 small groups) based on PC1 and PC2, with a cumulative value of 45.62% in PC1 and 31.05% in PC2. This is in accordance with the grouping based on the phylogenetic tree. Clade I only consisted of *D. corniculata*, clade II *D. pentaphylla*, Clade III consisted of *D. pectinata*, *D. Hymenophylloides*, *D. repens*, *D. trichomanoides*, *D. heterophylla*, *D. denticulata*, *D. solida* var. *solida*, *D. divaricata* and *D. repens*. Differences in genetic distance cause the separation of clades in clusteri analysis using PCA. Thus grouping can explain genetic variations based on nitrogenous bases found in species.

The reconstruction of phylogenetic trees and PCA plots based on the *trnL-F* marker in *Davallia* species has a high ability to explain the classification position to the species level. The resulting genetic variation has low homology, which is suitable for use in phylogenetic analysis and PCA at the species level. This can be demonstrated from the 7 subclades (Figure 3). *Davallia denticulata* and *D. solida* var. *solida* with bootstrap value of 81% collected from several locations in Sumatra and the Mentawai Islands were clustered together but separated by branching. The difference in species grouping was caused by a change in nitrogen base, as shown in Table 2. Meanwhile, *D. divaricata* and *D. heterophylla* are in the same clade as *D. hymenophylloides*, *D. repens*, which is in the same clade. This is due to many sequences with the same percentage reaching 80% (Table 2; Figure 2). These phylogenetic tree reconstruction results support the previous grouping proposed by Tsutsumi and Kato (2005).

Mutations in *Davallia* are caused by changes in nitrogenous bases both at the intraspecies and interspecies levels. Mutations cause differences in phenotypic characters encoded by genes as a form of adaptation to different environments. Intraspecies mutations are observed in changes in nitrogen bases in the same population, whereas interspecies mutations were found in different species. In this study, intraspecies mutations were observed in 5 *Davallia* species, including *D. denticulata*, *D. pectinata*, *D. repens*, *D. solida* var. *solida*, and *D. trichomanoides*, while interspecies mutations were also observed in 5 *Davallia* species, namely *D. corniculata*, *D. divaricata*, *D. heterophylla*, *D. hymenophylloides*, and *D. pentaphylla*. Data on interspecies mutations were obtained based on observations of nitrogenous bases with the outgroup (*O. articulata*). Based on the results of mutations in 10 species of *Davallia*, it was found that the difference in the nucleotide base sequence was less than 1%. In contrast, the difference in nitrogen bases reached 4% in interspecies. The difference in the percentage of nitrogen bases was obtained based on comparison of base pair (bp) differences among species. This indicates that using *trnL-F* IGS collection markers correctly identifies a species that generally has a higher mutation fee inside the upper species taxon than the decreased species taxon (Li et al. 2011).

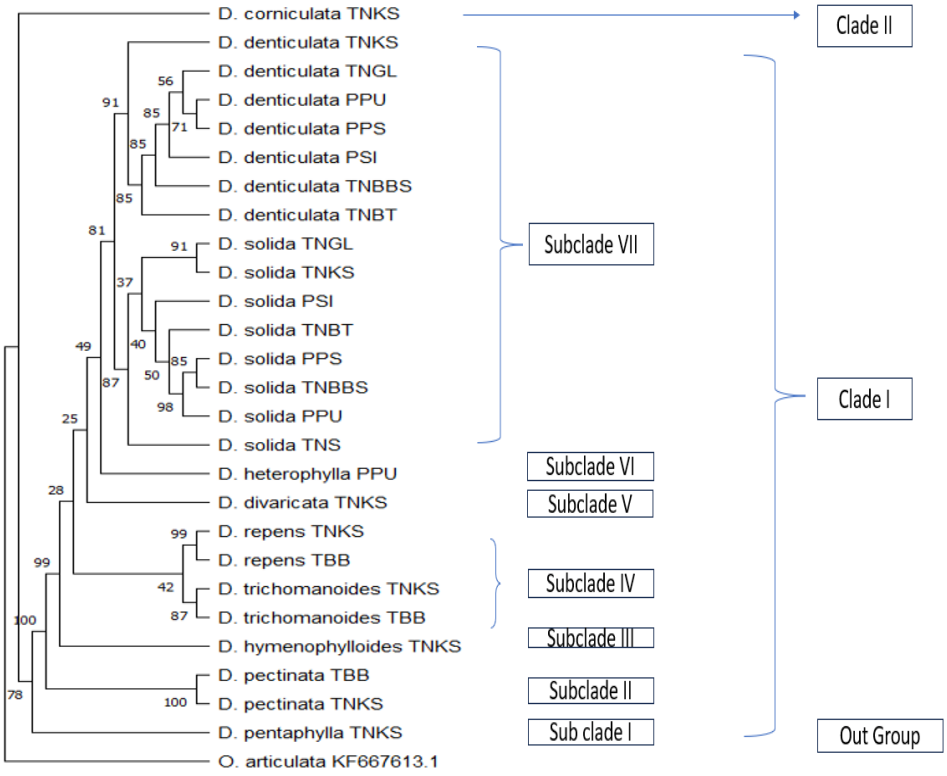

Figure 3. Phylogenetic tree based on sequences *trnL-F* IGS of genus *Davallia* and outgroup as a result of sequence data reconstructions using Maximum Parsimony method with 1000 replicates bootstrap

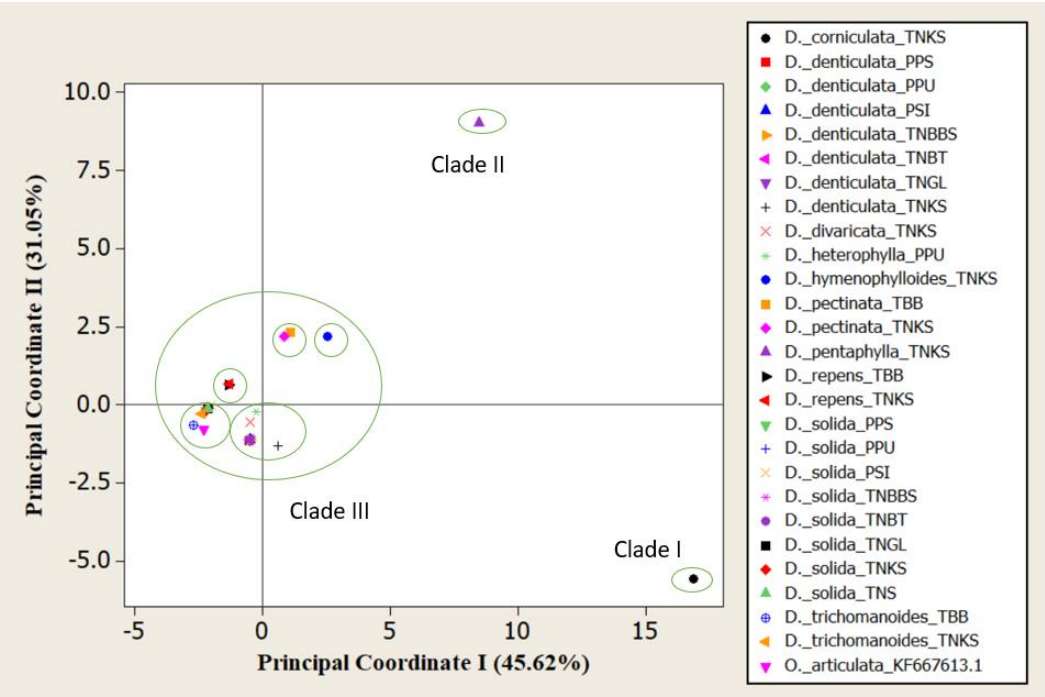

Figure 4. Grouping based on Principal Component Analysis (PCA) of ten *Davallia* species in Sumatra and Mentawai Islands. I. *D. Corniculata* (OQ077741), II. *D. Pentaphylla* (OQ077754), III. *D. pectinata* ((OQ077752-OQ077753), *D. hymenophylloides* (OQ077751), *D. repens* (OQ077755- OQ077756), *D. trichomanoides* (OQ077765- OQ077766), *D. heterophylla* ((OQ077750), *D. denticulata* (OQ077742-OQ077748), *D. solida* var. *solida* ((OQ077757- OQ077764), *D. divaricata* (OQ077749), and *D. repens* (OQ077755- OQ077756).

Morphological identification of *D. Denticulata*, and *D. solida* var. *solida* show many similarities in morphological characteristics along with the form of the rhizome, size, and shape of the leaf shape. However, there are differences in the length of the sorus between the accessions on Sumatra and Mentawai Islands. It shows that morphological similarities (cryptic species) occur in *Davallia* species. Cryptic species is a term for two or different species which might be morphologically comparable so that this hassle may be solved using a molecular method (Lukhtanov 2019). Hence, the accession of *D. denticulata* and the accession of *D. solida* var. *solida*, originating from Sumatra and the Mentawai Islands, are still classified because of the identical species (conspecific species). Morphological variations were additionally determined in *D. trichomanoides* with *D. pentaphylla*. Those two species are in exclusive clades based on the order of their nitrogenous bases and the usage of the IGS *trnL-F* marker. This could give an explanation for that these species have exclusive morphological facts and molecular statistics, that reason proving that the two species are distinct.

The separation of populations originating from distinctive national Parks in Sumatra and Mentawai Islands is due to differences in geographical vicinity. This makes it viable if all those populations had been reproductively remoted, resulting in genetic variations between those regions. The joining of molecular phylogeny groupings between populations of a *Davallia* species on Sumatra and Mentawai Islands indicates that those populations are intently related even though these populations are separated via an extensive ocean strait. The unification of these corporations is likely associated with the separation and unification between Sumatra and Mentawai Islands in the past, which allowed one species to revel in a couple of invasions (Gaskin et al. 2018). Another elements that also support genetic similarity between *Davallia* populations in Sumatra and Mentawai Islands is the time of *Davallia* divergence, taking into consideration the envisioned ages of the crowns and stems, as well as the fossil file in Davalliaceae, so this genus has been isolated from different genera in Davalliaceae for the reason that overdue Miocene (Liu and Schneider 2013). At the same time, Sumatra and Mentawai Islands had been separated 500,000 years in the past (Verstappen 1973), so it has miles suspected that the *Davallia* species in Mentawai Islands have a common ancestor with the species from Sumatra.

In conclusion, the analysis and reconstruction of the molecular phylogeny tree of *Davallia* (Davalliaceae) from Sumatra and Mentawai Island (Indonesia) based on the *trn*L-F intergenic spacer (IGS) sequence resulted in a monophyletic phylogenetic tree topology. The tree consists of two clades with 7 subclades. The *D. corniculata* included in clade II shows the longest branching compared to other branches and is closest to the outgroup, so it is suspected that this species is the most primitive. This approach also obtained the same results as the clustering using PCA analysis. This result indicates that the *trn*L-F IGS sequence marker accurately identifies in the species level of *Davallia*.

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