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Short Communications

Genetic Diversity of *Dicranopteris* and *Sticherus* from Rokan Hulu, Riau Based on ISSR Marker

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ABSTRACT
Genetic diversity of eleven taxa consisted of *Dicranoperis speciosa, D. curanii,* and *D. linearis* with seven varieties and *Sticherus truncatus* with two varieties from Rokan Hulu, Riau was analyzed using ISSR markers generated from 10 primers. Nine out of ten ISSR primers produced a high level of polymorphism, with six of them showed 100% polymorphism. The genetic similarity was calculated using Jaccard's similarity coefficient, and cluster analysis using the Unweighted Pair-Group Method with Arithmetic Mean. The result showed that the genetic similarity of the eleven taxa under study ranged from 0.377 to 0.627 indicated a moderate level of genetic diversity and the clusters did not separate *Dicranopteris* from *Sticherus*.

Indonesia is a mega biodiversity country with a high variety of plant taxa including Pteridophytes. The Pteridophytes taxonomical study by Van Steenis (1959) in the Malesian region was the main reference for the fern diversity in Indonesia (Go et al. 2012). There were a number of taxonomic studies on Pteridophytes diversity in Indonesia such as those of Adjie & Lestrari (2011) who reported 54 species of Pteridophytes from Bali, Arini & Kinho (2012) with 41 species from 19 families from North Sulawesi, and Sofiyanti et al. (2020) who found 23 fern species from Meranti Riau.

Gleicheniaceae is an ancient Pteridophyte with the oldest fossil evidence found during the Carboniferous period (Tryon & Tryon 1982). There are six genera of Gleicheniaceae in the world, namely *Dicranopteris* Bernh., *Diplopterygium* (Diels) Nakai, *Gleichenella* Ching, *Gleichenia* Sm., *Sticherus* C. Presl, and *Stromatopteris* Mett. (Smith et al. 2006; PPG I 2016). According to Van Steenis (1959), three genera of Gleicheniaceae found in the tropics and subtropics were *Stromatopteris* (1 species), *Dicranopteris* (10 species), and *Gleichenia* (50 species, which some species were taxonomically revised to *Sticherus*). The diagnostic characters of *Sticherus* are the young organ parts are protected by peltate scales and stellate hairs, sori arranged into two to five sporangia, and simple branching venation. Meanwhile, the distinguishing characters of *Dicranopteris* are the young organs are protected by branched

hairs in various forms, no scales, sori composed of 8-15 sporangia or more, and the venation is branched at least twice. So far, studies on the diversity and taxonomic relationships of Gleicheniaceae in Indonesia were mainly based on morphological characters, and there is no information on the genetic diversity of this taxon based on molecular markers.

Inter Simple Sequence Repeat (ISSR) is a molecular marker commonly used to analyze the genetic variation of closely related species (Zietkiewicz et al. 1994). DNA fingerprinting patterns generated using ISSR markers have been used in taxonomic studies of ferns such as reported by Vidyashree et al. (2019) who evaluated the taxonomy of 19 fern species in India. Other studies on Pteridophyte using ISSR to determine genetic diversity were done by Korpelainen et al. (2005) on *Adiantum*, Wang et al. (2012) on *Alsophila spinulosa*, and Fernando et al. (2015) on *Asplenium scolopendrium* var. *americanum*. In this study, ISSR marker was used to reveal genetic diversity and taxonomic relationships of *Dicranopteris* (three species, one of them with seven varieties) and *Sticherus* (one species, *S. truncatus* with two varieties) as shown in Table 1. Genomic DNA was extracted from 50 mg of fresh leaves using Geneaid Genomic DNA Mini Kit (Plant) following the procedure of the manufacturer. The DNA samples were stored at -20°C before the PCR amplification process. The PCR was performed following Vidyashree et al. (2019) with modifications on the annealing temperature. A total of 10 primers were used in this study. The amplification of ISSR markers was performed in 25 µl volume reaction containing 2 µl DNA (0.5-2.0 ng), 2 µl of primer ISSR, 12.4 µl of DreamTaq™ Hot Start Green PCR Mix (DreamTaq Hot Start DNA polymerase, 2X DreamTaq Green Buffer, 0.4 mM dNTPs and 4 mM $MgCl_2$), and 8,6 µl water free nuclease.

Table 1. List of species and varieties used for ISSR analysis.

No.	Genus	Spesies
1	*Dicranopteris*	*Dicranopteris linearis* (Burm. *f.*) Underw. var. *altissima* Holttum
2		*D. linearis* var. *inaequalis* (Rosenst.) Holttum
3		*D. linearis* var. *linearis*
4		*D. linearis* var. *demota* Holttum
5		*D. linearis* var. *alternans* (Mett.) Holttum
6		*D. linearis* var. *tertraphylla* (Rosenst.) Nakai
7		*D. linearis* var. *subspeciosa* Holttum
8		*D. curranii* Copel.
9		*D. speciosa* (Presl) Holttum
10	*Sticherus*	*Sticherus truncatus* (Willd.) Nakai var. *involuta* Holttum
11		*S. truncatus* var. *truncata*

The amplification condition refereed to by Vidyashree et al. (2019) with modification on the annealing temperature after the optimation procedure. The reactions consisted of one cycle of initial denaturation at 94° C for 2 minutes, 35 cycles of amplification reactions consisted of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 30 seconds, and followed by a final extension at 72°C for 10

minutes. The amplification products were detected in 1.5% agarose gel in 1X TBE Buffer stained with ViSafe Green Gel Stain (10,000x in water) for 40 minutes at 100 voltage. The results were photographed under the blue light Transilluminator. The DNA fragments were manually scored as present (1) or absent (0) to generate binary data for cluster analysis. The cluster analysis was done based on Jaccard's similarity coefficients and Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) methods using MultiVariate Statistical Package (MVSP) program Version 3.22 (Kovach 2007). Nine out of ten ISSR primers used in this study produced a high degree of polymorphism ranged from 77.68% to 100%, and only one primer failed to produce amplification products (Table 2). The possibility that primer ISSR 816 failed to produce amplification products might be caused by several factors such as primer incompatibility, non optimum annealing temperature for this particular primer, and different requirements for PCR reaction especially the Mg^{2+} concentration (Ali et al. 2006; Pharmawati 2009; Mariana et al. 2011; Budiani et al. 2016). A total of 96 DNA fragments were generated from nine primers, with the number and size varied from 100 to 1,100 bp. In this study, six primers namely ISSR 845, ISSR 847, ISSR 851, ISSR 855, ISSR 859, and ISSR 888 produced 100 % polymorphisms. Representatives of DNA fingerprinting profiles were shown in Figure 1.

Most of the primers used in this study, with one exception for ISSR 816, showed a higher level of polymorphism than previous studies on ferns genetic diversity using ISSR markers (Vidyashree et al. 2019). A High level of polymorphism indicated the effectiveness of ISSR primers for the purpose of genetic diversity analysis. Other genetic diversity studies using ISSR as molecular markers have found high levels of polymorphism, such as those in *Adiantum* with 82% polymorphic bands (Korpelainen et al. 2005), six ornamental fern species with 71.26% polymorphic bands (Animasaun et al. 2018), and two endemic Lycophyte species, *Isoetes cangae* and *Isoetes serracarajensis* with 87% polymorphic bands (Santos et al. 2020).

The result of cluster analysis on 11 taxa of *Dicranopteris* and *Sticherus* based on ISSR marker was presented as a dendrogram in Figure 2.

Table 2. Results of ISSR marker amplification.

No.	Primer	Sequence	Fragment length (bp)	No. of bands	Polymorphic band (%)
1	ISSR 816	5'CACACACACACACACAT3'	0	0	0 (0 %)
2	ISSR 845	5'CTCTCTCTCTCTCTCTCTRG3'	310-900	7	7 (100 %)
3	ISSR 847	5'CACACACACACACACARC3'	200-900	14	14 (100 %)
4	ISSR 851	5'GTGTGTGTGTGTGTGTYG3'	230-600	8	8 (100 %)
5	ISSR 855	5'ACACACACACACACACYT3'	230-500	10	10 (100 %)
6	ISSR 857	5'ACACACACACACACACYG3'	100-750	13	12 (92.31 %)
7	ISSR 859	5'TGTGTGTGTGTGTGTGRTRC3'	150-550	12	12 (100 %)
8	ISSR 861	5'ACCACCACCACCACCACC3'	300-750	9	7 (77.78 %)
9	ISSR 862	5'ACCACCACCACCACCACC3'	350-740	7	6 (85.71 %)
10	ISSR 888	5'BDBCACACACACACACA3'	150-1100	16	16 (100 %)
		Total		96	

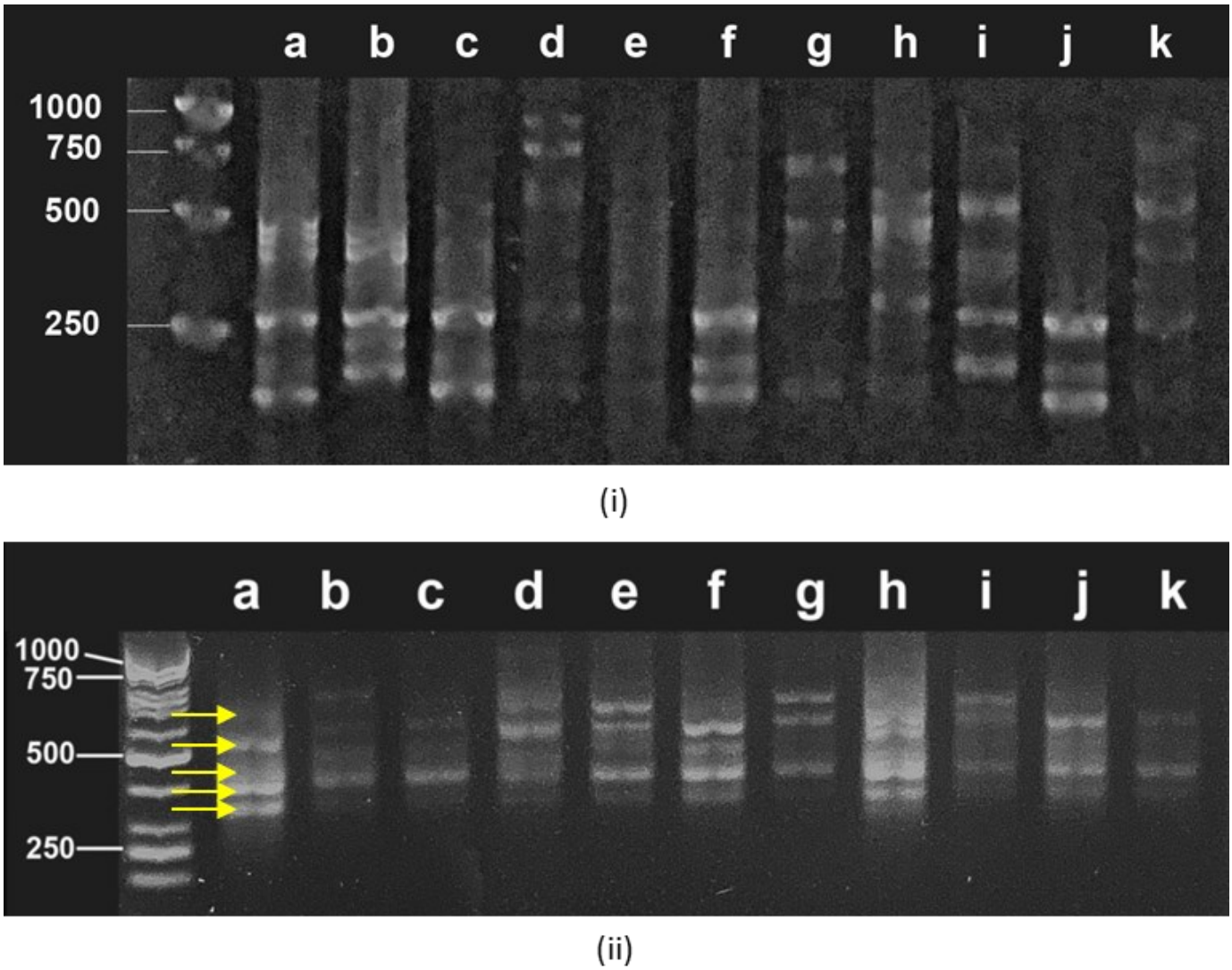

Figure 1. DNA fingerprinting profiles of *Dicranopteris* species and *Sticherus truncatus* showing polymorphisms from two primers: (i) primer ISSR 847 shows 100% polymorphic band, (ii) primer 862 shows 85,71% polymorphic band (yellow arrows indicate bands present in all samples with different intensities). **a** *D. linearis* var. *altissima*, **b.** *D. linearis* var. *inaequalis*, **c.** *D. curranii*, **d.** *S. truncatus* var. *involuta,* **e**. *D. linearis* var. *linearis*, **f**. *D. linearis* var. *demota*, **g**. *S. truncatus* var. *truncata*, **h.** *D. linearis* var. *alternans*, **i**. *D. linearis* var. *tertraphylla*, **j**. *D. speciosa*, **k.** *D. linearis* var. *subspeciosa*.

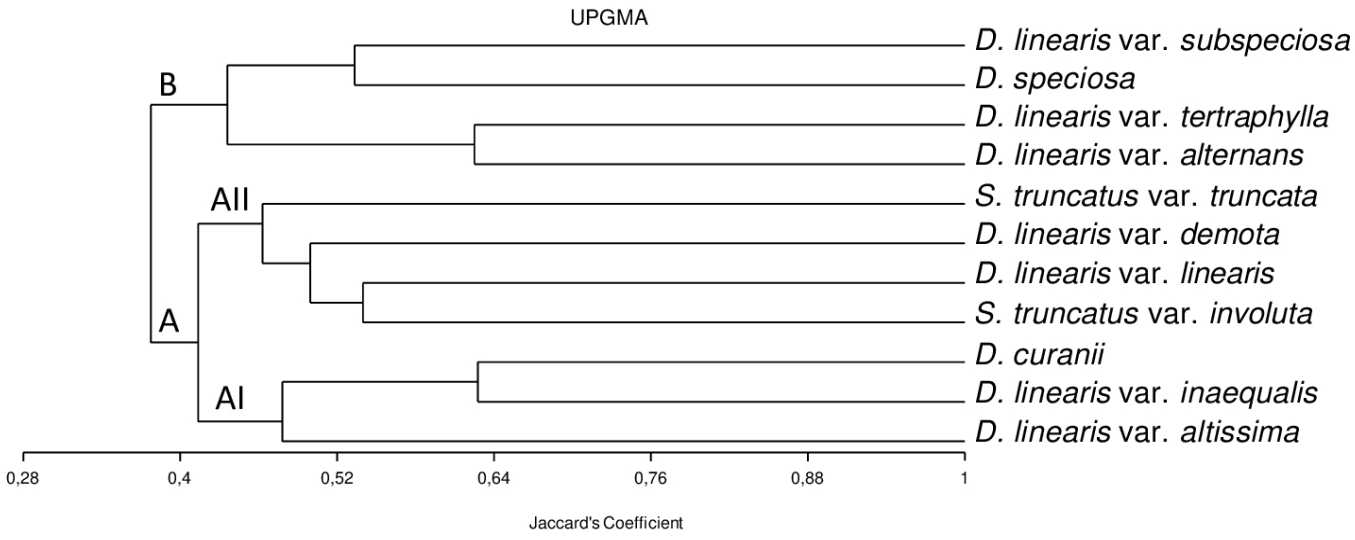

Figure 2. Dendrogram showing taxonomic relationships of *Dicranopteris* and *Sticherus* based on ISSR marker.

The dendrogram showed the formation of two main clusters namely clusters A and B, with a genetic similarity of 0.377. Cluster A was made up of seven taxa which were divided into two sub-clusters, whereas Cluster B was made up of four taxa. The genetic similarity of 11 Gleicheniaceae taxa from Rokan Hulu, Riau ranged from 0.377 to 0.627 based on the Jaccard similarity coefficient (Table 3). According to Rahayu et al. (2007), a genetic similarity of 0.29 indicated low diversity whereas 0.889 indicated high diversity. Andayani et al. (2016) also claimed that a value of 0.39 was considered as low, 0.70 was considered as moderate, and 0.92 was considered as high genetic diversity. Based on these previous studies using molecular markers, the genetic diversity of Gleicheniaceae in this study was moderate. The moderate genetic diversity found in the present study might be related to the growing nature of Gleicheniaceae that live in groups (Yatskievych 2018).

Another explanation for the moderate level of genetic diversity was the reproductive system of the taxa under study. Gleicheniaceae are plants that can reproduce sexually by spores and asexually by clonal propagation. Sexual reproduction is essential for dispersal and succession, whereas clonal reproduction plays a role in growth rate and territory coverage (Yang et al. 2020). *Dicranopteris* is known for its rapid clonal proliferation, and according to Russell et al. (1999), the clonal nature of this genus increases the rate of mating among gametophytes produced from the same sporophyte (intergametophytic selfing), which can reduce genetic diversity. A similar result was found in *Monimopetalum chinense* (Xie et al. 2011), *Elodea canadensis*, *Egeria densa* and *Lagarosiphon* (Lambertini et al. 2010), and *Nelumbo nucifera* (Mekbib et al. 2020) as clonal plants species live in groups with high levels of interpopulation interactions tend to have low genetic diversity due to inbreeding between populations. There were many factors causing a low level of genetic diversity in plants such as the inbreeding process in the population

Table 3. Similarity coefficient among *Dicranopteris* and *Sticherus*.

	A	B	C	D	E	F	G	H	I	J	K
A	1										
B	0.446	1									
C	0.509	0.627	1								
D	0.438	0.484	0.492	1							
E	0.482	0.458	0.441	0.54	1						
F	0.414	0.371	0.424	0.524	0.475	1					
G	0.269	0.375	0.318	0.478	0.475	0.435	1				
H	0.421	0.424	0.383	0.484	0.458	0.393	0.419	1			
I	0.415	0.393	0.4	0.459	0.429	0.386	0.323	0.625	1		
J	0.4	0.333	0.386	0.444	0.367	0.421	0.333	0.429	0.423	1	
K	0.321	0.237	0.286	0.333	0.316	0.321	0.283	0.404	0.489	0.533	1

A=*D. linearis* var. *altisima*; B=*D. linearis* var. *inequalis*, C=*D. curanii*, D=*S. truncatus* var. *involuta*, E=*D. linearis* var. *linearis,* F=*D. linearis* var. *demota*, G=*S. truncatus* var. *truncata*, H=*D. linearis* var. *alternans*, I=*D. linearis* var. *tetraphylla*, J=*D. speciosa*, K=*D. linearis* var. *subspeciosa.*

due to random genetic drift (Ellstrand & Elam 1993), limited gene flow, small population sizes, and fragmented populations also play a role in reducing the genetic variation of a species (Teixeira & Huber 2021).

In this study, seven varieties of *D. linearis* were found in different clusters in the dendrogram, in which four varieties were in the first cluster and the remaining three varieties were in the second cluster. This result indicating that *D. linearis* is a highly variable species. Based on morphological characters, previous research in the Malesiana region found high infraspecific diversity of *D. linearis*, such as Holttum (1957) who described 11 varieties, and Van Steenis (1959) recognized 13 varieties within the species. Plants that have a high level of infraspecific variation based on morphology might also have similar results in their genetic diversity analysis. Korpelainen et al. (2005) in their study on four species of *Adiantum* found differences in the ISSR characteristics on the taxa under study which indicated infraspecific variation. Other molecular genetic diversity studies by Dong et al. (2007) on *Ceratopteris pteridoides*, and Barker & Hauk (2003) on *Sceptridium dissectum* showed that samples from the same species were found in different clusters based on ISSR marker. These studies were, therefore, also indicated the infraspecific variability on ferns.

The result of cluster analysis did not separate Gleicheniaceae taxa based on genera. In this study, *Sticherus* was placed in the same cluster as *D. linearis* in sub-cluster AII. According to Animasaun et al. (2018), species with different phenotypes do not necessarily have different genetic traits. A study by Vidyashree et al. (2019) on 19 species from 13 families of ferns using ISSR marker showed that *Pteris acanthoneura* (Pteridaceae) were found in the same group with Nephrolepidaceae and separated from other Pteridaceae. Fernando et al. (2015) reported similar results on genetic analysis in which *Asplenium longissimum* was found in different clusters from other *Asplenium* species based on ISSR markers.

The genetic diversity of Gleicheniaceae in Rokan Hulu, Riau, was categorized as moderate based on analysis of the ISSR marker. This moderate level of genetic diversity of Gleicheniaceae might be due to this fern's clonal propagation nature in their reproductive system. The results of this study open up opportunities for further research in finding specific molecular markers to identify *Dicranopteris* and *Stricherus*. This study which showed evidence of genetic diversity on infraspecific level of these two genera also provided the basis for the conservation of ferns species as part of biodiversity in this country.

AUTHORS CONTRIBUTION

AAM collected plant samples, analyzed the data, and wrote the manuscript. RS designed the research, supervised all the processes from the field work to laboratory analysis, and wrote the manuscript.

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CONFLICT OF INTEREST

The authors state that they do not have any conflicts of interest. The authors are solely responsible for the article's content and writing.

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