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Genetic diversity based on SSR markers of 30 *Aeridinae* subtribe orchid genetic resources of Indonesian Ornamental Crop Research Institute, Cianjur, Indonesia

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Abstract. *Wegadara M, Dewanti M, Diningsih E, Rachmawati F, Sukma D, Sudarsono. 2022. Genetic diversity based on SSR markers of 30* Aeridinae *subtribe orchid genetic resources of Indonesian Ornamental Crop Research Institute, Cianjur, Indonesia. Biodiversitas 23: 2943-2956.* Advances in molecular biotechnology encourage molecular markers to identify plant diversity to become increasingly common. Using SSR markers, this study aimed to determine the genetic diversity and phylogenetic relationships of 30 accessions from five parental genera of *Aeridinae* orchids from the IOCRI genetic resource collection. Molecular characterization of 30 orchid subtribes *Aeridinae* genetic resources of IOCRI was carried out using 17 polymorphic SSR markers. The resulting binary data was then analyzed using the GenAlEX program to determine Na, Ne, Ho, He, and the PowerMarker V3.25 program to determine the values of MAF, D, and PIC. Molecular characterization using 17 SSR primers generated 240 amplified DNA fragments across 30 accessions, with the fragment sizes ranging from 56-4818 bp. Factorial analysis (PCoA) and phylogenetic tree construction clustered the 30 *Aeridinae* accessions into three major clusters. Accessions belonging to the *Vanda* genus are clustered into three clusters (Clusters I, II, and III). The *Vascostylis* is in Cluster I, along with 12 *Vanda* accessions. Two accessions of *Vanda*, two *Aranthera*, three *Arachnis*, and one *Aerides* genera are clustered into Cluster III. This study showed that interspecific hybrids among accessions of *Vanda* within Cluster I and Cluster II should be possible since they are genetically closely related. Intergeneric hybrids among the accession of *Vascostylis* and *Vanda*'s genera belonging to Cluster I, should also be possible. Furthermore, it should be possible to generate intergeneric hybrids among *Aerides*, *Arachnis*, *Aranthera*, and *Vanda* accessions within Cluster III. The generated data from this study should be helpful for future *Aeridinae* breeding activities intended to generate new hybrid varieties.

Keywords: *Terrestrial orchids*, *Orchidaceae*, PIC, phylogenetic analysis, SSR markers

Abbreviations: PIC: polymorphic information content, He: expected heterozygosity, Ho: observed heterozygosity, Na: number of alleles, Ne: number of effective alleles, MAF: major allele frequency, D: gene diversity, SSR: simple sequence repeats

INTRODUCTION

The *Orchidaceae* family, order *Asparagales* is the most abundant flowering plant worldwide, consisting of five subfamilies, 22 tribes, 736 genera, 28,000 species (Zotz 2013; Chase et al. 2015; Christenhusz and Byng 2016) and more than 30,000 known hybrids (Hew and Yong 2004; Zotz 2013; Chase et al. 2015; Christenhusz and Byng 2016). The subtribes *Adrorhizinae*, *Aeridinae*, *Angraecinae*, and *Polystachyinae*, make up the tribe *Vandeae* of the subfamily *Epidendroideae* (Dressler 1993; Chase et al. 2015). With 83 genera and 1,314 species, the subtribe *Aeridinae* has the most members (Chase et al. 2015), and it exists throughout Asia, Australia, and a tiny section of Africa (Hidayat et al. 2012). Tropical Asia and Australia are where the core diversity of subtribe *Aeridinae* (Dressler 1993). The five genera from the subtribe *Aeridinae* evaluated in this study include *Aerides*, *Arachnis*, *Aranthera*, *Vanda*, and *Vascostylis* since they are the core collections and breeding stocks of the Indonesian Ornamental Crops Research Institute (IOCRI).

Indonesia is a developing country with an economic focus on the agricultural sector (Sayifullah and Emmalian, 2018; Parmadi et al. 2018). Ornamental horticulture is one of the fast-growing agricultural sectors and contributes significant support to the Indonesian economy (Mutakabbir and Duakaju 2019; Zameda Igga et al. 2019). Orchids are one of the ornamental plants that have the economic potential to support the future Indonesian economy (Puspitasari et al. 2018; Harniati and Jamil 2020). The export value of orchids from Indonesia in 2020 was US$ 69,500, representing a 128% increase from the 2019 export value. The leading Indonesian orchid importers are Japan, South Korea, and Singapore (BPS 2020).

Commercial orchid varieties in the international markets include intra-specific, intra-generic, and closely related inter-generic hybrids, with the significant aim of producing new orchid types (Hartati 2019; Antonetti et al.

2021) as a result of changes in the genetic constitution through segregation and recombination (Marwoto et al. 2012; Liu et al. 2016). Hybrid varieties in orchids are desirable in the floriculture industry because they exhibit heterosis (Govindaraju 2019; Dwiati and Susanto 2021). However, breeding for commercial orchid hybrids exhibits many significant constraints because of the vast genetic differences among accessions (Hartati et al. 2019) and the diverse evolution of the orchid germplasm (Zhang et al. 2012; Guo et al. 2015). Therefore, the availability of genetic information and the evolutionary relationships among orchid accessions needed to form new orchid varieties are necessary. Moreover, diversity evaluation among accessions of orchid subtribe *Aeridinae* collections will benefit future research.

Unlike morphological characters, molecular markers are not affected by environmental changes. Molecular markers are more accurate and show higher polymorphism than morphological characters (Rajaram et al. 2019; Tang et al. 2021). DNA-based markers are helpful for various genetic studies (i.e., germplasm fingerprinting, genetic diversity, evolutionary studies) and for supporting breeding programs (i.e., gene tagging, marker-assisted selection, and marker-assisted back-crossing) (Surveswaran et al. 2018). The simple sequence repeats (SSRs) as highly variable plastome markers may help identify closely related species in the *Aeridinae* family, including several wild species and countless cultivars generated from interspecific or intergeneric hybrids (Kim et al. 2020).

Simple sequence repeats, also known as microsatellites, are tandemly repeated DNA sequences with two to six nucleotide repeat units and are common in most eukaryotic genomes (Pandey and Sharma 2012; Kumar et al. 2018). The SSR markers are desirable since they are abundant and uniformly distributed in the genome, highly polymorphic, and exhibit codominant alleles (Khodaeiaminjan et al. 2018; Kumar et al. 2018; Eser et al. 2019). Molecular markers, especially SSR, have been used to evaluate orchid genetic diversity (Cai et al. 2012; Kang et al. 2015; Yun et al. 2020).

However, molecular markers used to evaluate the genetic relationship and diversity of the *Aeridinae* subtribe have been limited (Lim et al. 2007, Phuekhvilai et al. 2009, Hidayat et al. 2012, Peyachoknagul et al. 2014, Zou et al. 2015). The SSR markers have been used in orchid research for the characterization of microsatellites in Mokara and the intergeneric hybrid from 3 genera (*Vanda*, *Ascocentrum*, and *Arachnis*); genetic diversity of endangered *Neofinetia falcata*; genetic diversity of the *Cymbidium goeringii*; and genetic diversity of the endangered orchid *Pelatantheria scolopendrifolia* (Peyachoknagul et al. 2014; Han et al. 2018; Noh et al. 2020; Yun et al. 2020). Research conducted by Hidayat et al. (2012) used DNA sequences of ITS region of nrDNA and matK of cpDNA to determine the phylogenetic relationship of the subtribe *Aeridinae*. Hidayat et al. (2012) divided the subtribe *Aeridinae* into four main clades with 11 subclades. The phylogenetic relationships of the subtribe *Aeridinae* have also been reconstructed using five DNA sequences (ITS, atpI-H, matK, psbA-trnH, and trnL-F) and grouped into ten monophyletic clades (Zou et al. 2015). Therefore, further investigation on using SSR markers for characterizing the *Aeridinae* subtribe germplasm collections should be helpful. This study aims to characterize the genetic diversity of 30 *Aeridinae* subtribe accessions of the Indonesian Ornamental Crop Research Institute (IOCRI) using 17 simple sequence repeat (SSR) primers, estimate their genetic diversity, and construct a phylogenetic tree based on their genetic distances. The generated information should be valuable for supporting IOCRI's future *Aeridinae* breeding programs and developing new *Aeridinae* orchid varieties.

MATERIALS AND METHODS

Plant materials and DNA isolation

The plant materials used in this study are part of the *Aeridinae* genetic resources available in the IOCRI, West Java, Indonesia. The evaluated 30 *Aeridinae* accessions consist of the representative *Aerides* (one accession), *Arachnis* (three accessions), *Aranthera* (two accessions), *Vascostylis* (one accession), and *Vanda* (23 accessions) (Table 1).

Either young leaves or flower petals were harvested and sent to the Integrated Breeding Laboratory of IOCRI for DNA extraction using protocols as described by Lim et al. (2007). Total DNA was isolated from 0.1 g of either young leaf or flower samples using the GeneJET™ Plant Genomic Purification Mini Kit (Thermo Scientific, USA) with the addition of 0.7 g polyvinylpolypyrrolidone (PVPP). The isolated DNA was resuspended in 50-100 µL of aqua bidest and checked for their quantity and quality using a DNA Spectrophotometer (Implen, Germany). A ratio of A260 and A280 readings was used to estimate the quantity and purity of the isolated DNA (Sambrook et al. 1989).

Genotyping using Simple Sequence Repeat (SSR) markers

A total of 17 SSR primer pairs, previously used for *Aeridinae* genetic characterization (Lim et al. 2007, Phuekhvilai et al. 2009, Peyachoknagul et al. 2014) were used for genotyping the IOCRI's *Aeridinae* accessions (Table 2). The final volume of the PCR reaction was 25 µL, consisting of DNA template (2 µL), DreamTaq PCR master mix (12.5 µL) (Thermo Scientific, USA), forward and reverse primer (1 µL of 0.4 M), and 8.5 µL ddH_2O. The PCR amplification was carried out in a Gradient PCR Thermal Cycler (SensoQuest, Germany) using the following settings 94ºC for 3 minutes of initial denaturation, 35 cycles of denaturation at 94ºC for 30 seconds, annealing at appropriate temperatures (depending on the primers used) for 1 minute, and primer extension at 72ºC for 30 seconds, followed by a final primer extension cycle at 72ºC for 5 minutes (Peyachoknagul et al. 2014). The PCR amplified DNA products were fractionated using the Automated Parallel Capillary Electrophoresis (CE) System (QIAxcel Advanced, Qiagen, Germany) Fragment

Analyzer. The 15 and 5,000 bp standard and 100-2,500 bp DNA ladders were used as size DNA control.

Scoring and data analysis

The amplified fragment size was automatically recorded in the Automated Parallel Capillary Electrophoresis (CE) System (QIAxcel Advanced, Qiagen, Germany) Fragment Analyzer apparatus. The data was then stored in MS Excel software for further analysis. All DNA fragments recorded for each accession were assigned a score of 1 (DNA fragment present) or 0 (DNA fragment absence). Dominant marker data can cause estimation bias by overestimating parameters by 5%, especially with small sample sizes (Lynch and Milligan 1994). To account for this bias, we followed the method of Lynch and Milligan (1994) to remove loci with a band frequency higher than 1-(3/N), with N: number of individuals sampled. Since we used 30 accessions, PCR amplified DNA occurring in only one or two accessions is removed. The number of alleles (Na) and the number of effective alleles (Ne), observed heterozygosity (Ho), and expected heterozygosity (He) (per population and primer) were calculated by the GenAlEX version 6.5 program (Peakall and Smouse 2012). The ability of a locus to differentiate genotypes was measured based on the value of gene/marker diversity (D), polymorphism information content (PIC), and Major Allele Frequency (MAF), which were calculated using the PowerMarker V3.25 program (Liu and Muse 2005). The simple matching method was used to calculate the dissimilarity index of allelic data, and the weighted Neighbor-Joining method was used to construct the phylogenetic tree. Both dissimilarity index and phylogenetic tree were determined using DARwin software version 6.0.21 (Perrier and Jacquemoud-Collet 2014).

Table 1. List of the evaluated Indonesian Ornamental Crop Research Institute (IOCRI) *Aeridinae* orchid subtribe collections in this study

Genera	Accession names	Accession codes	Ploidy and cross-type
Vanda	*Ascocenda* Salva Dela Pena x *Ascocenda* Lauraine Paull	ASDLP	Diploid hybrids
Vanda	*Vanda sanderiana* x *Vanda* President Quirino No 1	VsVQ1	Diploid hybrids
Vanda	*Vanda insignis*	Vi	Diploid species
Vanda	*Vanda* Alice Fukunaga x *Vanda sanderiana* "Guan Poe" (H1763 No 1)	VsAFs	Diploid hybrids
Vanda	*Vanda* Chiginori	VC	Diploid hybrids
Vanda	*Vanda limbata*	Vl	Diploid species
Arachnis	*Armodurum sulingii*	AS	Diploid species
Vascostylis	*Vascostylis* Pine Rivers	VPR	Diploid intergeneric hybrids
Aranthera	*Aranthera* James Storii	AJS	Diploid intergeneric hybrids
Aranthera	*Aranthera* Ladda Gem	ALG	Diploid intergeneric hybrids
Arachnis	*Arachnis* Maggie Oei	AMO	Diploid hybrids
Vanda	*Ascocentrum miniata*	Am	Diploid species
Vanda	*Vanda tricolor*	Vt	Diploid species
Arachnis	*Arachnis flos-aeris*	AFa	Diploid species
Vanda	*Vanda* Udom Gold x *Vanda* Doctor Anek Fuchs Gold	VUGDAF	Tetraploid hybrids
Vanda	*Ascocenda* Lauraine Paull x *Ascocenda* Gua Chia Long (V.92)	ALPGCL	Diploid hybrids
Vanda	*Vanda* Doctor Anek x *Vanda tricolor*	VDAt	Diploid hybrids
Vanda	*Vanda* Fuchs Delight x *Vanda lombokensis*	VFDl	Diploid hybrids
Aerides	*Aerides odorata*	Ao	Diploid species
Vanda	*Vanda* Adisak x *Ascocenda* Tubtim Velvet	VAATV	Tetraploid hybrids
Vanda	*Vanda* Robert's Delight 'Ink Blue'	VRDib	Diploid hybrids
Vanda	*Vanda* Robert's Delight 'Pink' PCH No. 15	VRDp15	Diploid hybrids
Vanda	*Vanda* Pachara Delight	VPD	Diploid hybrids
Vanda	*Vanda* Madame Rattana	VMR	Diploid hybrids
Vanda	*Vanda sanderiana*	Vs	Diploid species
Vanda	*Vanda* Emma Storie	VES	Diploid hybrids
Vanda	*Ascocenda* Guo Chia Long x (*Vanda* Christoper Bull x *Ascocenda* Yipsum Wah) No Y114	AGCL114	Diploid hybrids
Vanda	*Vanda* Robert's Delight 'Black'	VRDb	Diploid hybrids
Vanda	*Vanda* Golamco's Blue Magic	VGBM	Diploid hybrids
Vanda	*Vanda tricolor* x [(*Vanda* Patao x *Vanda* Jenny Hashimoto) x *Ascocenda* Peggy Foo] No 21	VtAPF21	Diploid hybrids

Table 2. List of simple sequence repeat (SSR) primer pairs used to generate SSR markers in this research, primer sequences, Tm, and the generated amplified PCR product numbers and size ranges

Primer code	Primer sequences (5′-3′)	Repeat sequences	Ta (°C)	PCR product fragments	
				Number	Size ranges (bp)
C32[1]	F:AATGGACCTTCTTTGCATTAC R:ATTACCGTTCATTTCTGGTGC	(GT)40(GA)27	46	7	100-446
C106[1]	F:AAGTCTAGCTTTTGGTTGAGG R:ATCGATGGTTTGTTCTTCTAGC	(TA)5(GT)45(GA)25	44	17	100-4811
C208[1]	F:TCATTGATGTTGGGAGCCTAA R:CTTGCCCTCTATCTTTCTCTT	(TA)3(GT)42(GA)10	50	15	101-418
C225[1]	F:AGAACTAGATGACTTCAAAACG R:GAACTCAGAAAAATTACCGCG	(GT)6(GA)24	47	6	48-379
C268[1]	F:TGGAAATGCATGTTGCCCGA R:ACTGAGTGACCTTGGAAGAC	(GT)17(GA)39	46	24	117-4818
C359[1]	F:CTTTGAGTAATGTCTCTCAGTG R:CCCTCACGCACTCTCTACC	(GA)15(GT)15	45	12	100-523
MOK26[1]	F:AGAATGAGGGAGGTATAGGG R:TGCCTTGGATGTGCGTTCG	(CCT)17	52	33	102-997
MOK29[1]	F:TTCAGCGTTTCCATGTCGAAG R:AGTAAAGCCGCCATCTTGG	(GA)13	52	6	157-215
MOK62[2]	F:AGAGTGAAGAGAGTGTTGG R:GGACTGTAAACTTCATGAGC	(GA)18	52	9	101-1102
MOK103[2]	F:TCTAGACATGTTTGAGAGGTGC R:TTACTCTTCCACTCTTCCATCC	(GA)34	50	14	101-450
MOK107[2]	F:CGCCCAACGAATAGAATGTTGG R:ACTATCTTCCTTACTCTTGCCCTC	(GA)22	56	14	102-4728
VMJ05[3]	F:GGGGGCTGCCAGAGTTG R:CCTTCTTTGATGGCTGTAGTCG	(CT)8	55	7	125-422
VMJ08[3]	F:TATCGCAGGAGGGAAAGTGTTC R:AGGAGAGGGTGGGGAATGG	(GA)7 (GT)7	55	22	100-1041
VMJ09[3]	F:ACTCTTCCTGCATAATAAAAACCA R:ACAAGTCACCCAAGGAATAGAAG	(CT)21	55	5	163-229
VMJ10[3]	F:TTACAGCTAGCGAGGAGGAAGA R:GCCATAATGAAGAAAGCAAGACA	(AG)17	55	19	101-447
VMJ14[3]	F:ATACCGGAGCAAGATAAGAAGATG R:ACCACTACAAGCCCCTCCAC	(GA)3 GTG AGT(GA)9	55	20	100-359
VMJ31[3]	F:CCTATGCGTGTGAATCAAATGG R:GGGGGTGTGAGGGGGTAAT	(CT)11	55	10	103-499
Total				240	
Average				14.1	

Note: [1]Phuekvilai et al. (2009), [2]Peyachoknagul et al. (2014), [3]Lim et al. (2007)

RESULTS AND DISCUSSION

PCR amplified SSR fragments

The results showed that the 17 SSR primers generated 240 amplified DNA fragments across 30 accessions, with the fragment sizes ranging from 56-4818 bp (Table 2). The number of amplified DNA fragments per primer ranged from five (VMJ09 primer pairs) to 33 (MOK26) (Table 2) and with an average number of 14.1 amplified DNA fragments. The number of amplified DNA fragments obtained using the evaluated SSR primer pairs in this study is higher than those reported by Lim et al. (2007), Phuekvilai et al. (2009), and Peyachoknagul et al. (2014).

In their studies using *Vanda* species, *Vanda* hybrid and intergeneric hybrid derived from Rhyncho *Vanda* (Rhynchostylis x *Vanda*) and Aranda (*Arachnis* x *Vanda*) and six SSR primer pairs (VMJ 05, VMJ 08, VMJ 09, VMJ 10, VMJ 14 and VMJ 31), Lim et al. (2007) obtained DNA amplified products ranged from 1-19 fragments per primer pairs. On the other hand, using 30 accessions of the *Aeridinae*, we obtained DNA amplified products ranging from 5 to 20 fragments for the same SSR primer sets. Moreover, in their studies using *Vanda* hybrid, *Vanda* species, and bigeneric *Vanda* and eight SSR primers (C32, C106, C208, C225, C268, C359, MOK 26, and MOK 29), Phuekvilai et al. (2009) obtained DNA amplified products ranged from 3-9 fragments per primer, while in our study there were 6-33 DNA fragments. Meanwhile, Peyachoknagul et al. (2014) used *Mokara*, *Vanda*, *Rhynchostylis*, *Aranda*, *Kagawara*, *Phalaenopsis Rhyncho Vanda*, *Renanstylis*, *Rhynchorides*, and a hybrid between *Arachnostylis* x *Ascocenda* resulted in DNA amplified products ranged from 3 to 19 alleles.

Han et al. (2018) evaluated the *Neofinetia falcata* species-an endangered orchid in Korea, using nine SSR primer pairs and showed ranging from 2 to 5 PCR amplified DNA fragments. Furthermore, an evaluation of the *Pelatantheria scolopendrifolia* orchid using microsatellite markers identified 18 SSR marker loci that could distinguish accessions of this species, and the primer sequences were deposited in the NCBI Genebank DNA database (accession no. MN592878-MN592895). The

amplified DNA fragments with the number of bands generated using the 18 SSR marker loci ranged from 2 to 9 fragments (Yun et al. 2020).

A representative sample of amplified DNA fragments among 11 *Aeridinae* subtribe orchid accessions from IOCRI genetic resources was amplified using the C268 SSR primer pairs. Their identified fragment sizes are presented in Figure 1. In figure 1, the identified PCR amplified DNA fragments using C268 SSR primer pairs ranging from 3 to 9.

Differences in the number of amplified DNA fragments using the evaluated SSR markers between this study and the previous ones (Lim et al. 2007, Phuekvilai et al. 2009, and Phuekvilai et al. 2009) may be due to the different samples used and the fragment separation methods. The study evaluated subtribe *Aeridinae* from five genera (*Aerides*, *Arachnis*, *Aranthera*, *Vascostylis*, and *Vanda*), interspecific and intergeneric hybrids. The DNA fragment separation method used the Automated Parallel Capillary Electrophoresis (CE) System (QIAxcel Advanced, Qiagen, Germany) Fragment Analyzer. In previous reports, Phuekvilai et al. (2009) and Phuekvilai et al. (2009) used polyacrylamide gel electrophoresis (PAGE), Lim et al. (2007), and Yun et al. (2020) used ABI 3730 XL, and Han et al. used QIAxcel for SSR fragments separation.

SSR polymorphism and genetic diversity

At the molecular level, genetic diversity in plants is estimated by the average value of the following parameters, such as estimated allele frequencies (p and q), the allele (Na) and effective allele (Ne) numbers, expected (He) and observed (Ho) heterozygosity, major allele frequency (MAF), gene diversity (D), and the polymorphic information content (PIC). Those parameters calculated among 30 *Aeridinae* accessions are presented in Tables 3 and 4. The estimated allele frequencies for the presence and absence of certain DNA is 0.62 (p: presence) and 0.38 (q: absence). The number of amplified DNA (Na: loci number) for each primer varied between 2-6, with an average value of 5.65 and the number of effective loci (Ne) for each primer ranged from 5.57-6.74, with an average value of 6.45. Meanwhile, the expected heterozygosity (He) ranged from 0.44-1.10 with an average value of 0.89, and the observed heterozygosity (Ho) was from 0.35-1.25 with an average value of 1.09.

Genetic diversity information in orchids is essential for orchid breeding and genetic improvement (Swarup et al. 2021). High genetic diversity facilitates orchid breeders in selecting the desired traits (George et al. 2020), and it is also essential, especially in association with unpredictable events because of climate change. Diverse genetics serve as germplasm collections hosting diverse traits, including tolerance to different biotic and abiotic stresses (Bhandari et al. 2017). According to Yun et al. (2020) inability to manage resources and population distribution could reduce genetic diversity and be disadvantageous for future cultivar development.

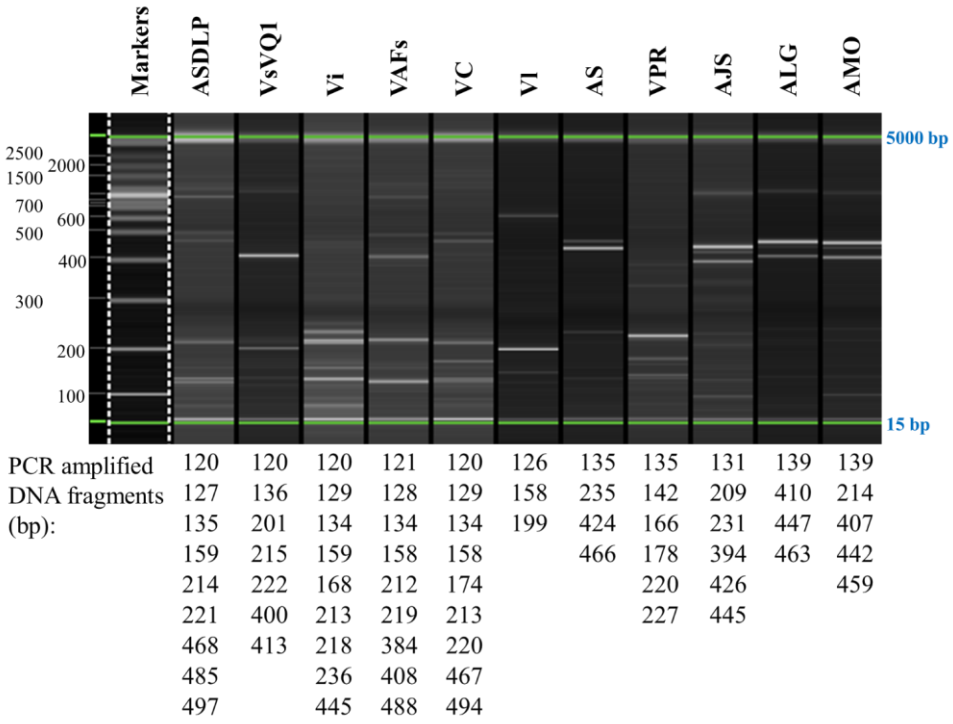

Figure 1. The PCR amplified DNA fragments from representative 11 accessions of the *Aeridinae* subtribe from the Indonesian Ornamental Crop Research Institute (IOCRI). ASDLP: *Ascocenda* Salva Dela Pena x *Ascocenda* Lauraine Paull, VsVQ1: *Vanda sanderiana* x *Vanda* President Quirino No 1, Vi: *Vanda insignis*, VAFs: *Vanda* Alice Fukunaga x *Vanda sanderiana* "Guan Poe" (H1763 No 1), VC: *Vanda* Chiginori, Vl: *Vanda limbata*, AS: *Armodurum sulingii*, VPR: *Vascostylis* Pine Rivers, AJS: *Aranthera* James Storii, ALG: *Aranthera* Ladda Gem, AMO: *Arachnis* Maggie Oei. Markers: DNA fragment size 100-2500 bp

Major allele frequency (MAF) across 30 *Aeridinae* subtribe ranged from 0.68 to 0.86, with a mean value of 0.80. The amplified DNA fragment occurring in all accessions is a common allele. Across the 30 *Aeridinae* subtribe accessions, the highest MAF was observed for the VMJ08 primer (0.86), while the lowest was for the VMJ05 primer (0.68). The lower the allele frequency, the rarer the allele appears in a particular locus. In the 30 *Aeridinae* subtribe accessions, many rare alleles are observed among most loci amplified from the evaluated SSR primers.

A higher number of allele diversities at one locus implies higher polymorphism. It is indicated by a higher value of PIC, whereas PIC describes genetic variation within a population under test (Gholami et al. 2021b). The results of this study indicated that the mean allele frequency is high, which further indicated that the alleles in the population are polymorphic. The molecular markers are helpful if they are highly polymorphic (Zhang et al. 2018; Gholami et al. 2021a) and can differentiate the evaluated accessions. The more polymorphic loci, the more informative the markers are for genetic evaluation since they indicate more genetic changes in the evaluated plants (Tikendra et al. 2019; Aloysius et al. 2020; Nugroho et al. 2020). Allele diversity (D) information for each locus was from 0.24 to 0.40, with a mean value of 0.30. VMJ05 had the highest allele diversity (0.40), while VMJ08 primer had the lowest allele diversity (0.24). VMJ05 data indicate that this primer has many diverse alleles in the tested population, and there are more observed alleles generated with the primer.

The polymorphic information content (PIC) measures the level of polymorphism at each locus. Botstein et al. (1980) suggested that the Polymorphism Information Content (PIC) value was used as a standard in developing PCR amplified DNA-based genetic markers. Botstein et al. (1980) grouped the PIC values into three classes: PIC>0.5 is very informative, PIC 0.25-0.5 is informative, and PIC<0.25 is less informative. The estimated PIC at each locus varies from 0.21 (VMJ08) to 0.31 (VMJ 05), with an average value of 0.25. According to the Botstein et al. (1980) grouping, the PIC observed in this study is grouped as moderate. However, eight SSR primers evaluated showed PIC values more significant than 25%, categorized as informative (C32, C208, C268, C359, MOK26, MOK29, VMJ05, and VMJ09, Table 3). The other nine primers were classified as uninformative with the PIC values less than 25% (C106, C225, MOK62, MOK103, MOK107, VMJ08, VMJ10, VMJ14, and VMJ31).

Lim et al. (2007) reported that the VMJ08 was the most informative SSR primer they evaluated. Meanwhile, the C106 and C225 SSR primers were very informative in a study reported by Phuekhvilai et al. (2009), and the C32 SSR primers did not generate any PCR product. Moreover, Peyachoknagul et al. (2014) showed that MOK 62 and MOK 103 SSR primers were polymorphic in all *Aeridinae* genera. Our study using 30 *Aeridinae* subtribe accessions showed different results since the PIC for C32 was >0.25, while the PIC for C106 and C225 were <0.25. Moreover, our results indicated that VMJ05 and VMJ09 were the most informative SSR primers for evaluating 30 *Aeridinae* subtribe accessions, and C106 primers were not informative for evaluating *Vanda* terete genetics.

The difference in the PIC value among different studies may be due to the sample differences in the current and the previous evaluations. The PIC values shown by the eight informative loci are similar to those reported for *Cymbidium goeringii* (Lee et al. 2020) and *Pelatantheria scolopendrifolia* (Yun et al. 2020). The very informative loci, with a PIC >0.5, are essential markers for the population genetic diversity analysis (Gholami et al. 2021a), and they can differentiate genotypes based on the known allele variations (Kaki et al. 2020). Therefore, the eight informative loci evaluated in this study (C32, C208, C268, C359, MOK26, MOK29, VMJ05, and VMJ09) can be effectively used to evaluate the genetic diversity of the *Aeridinae* subtribe collections of IOCRI.

The previous reports have indicated a relatively limited number of polymorphic SSR markers for the *Aeridinae* subtribe collection. The previously reported genetic analysis for the *Aeridinae* subtribe using molecular markers includes those reported by Yun et al. (2020) for the genetic diversity of *P. scolopendrifolia*, Kim et al. (2020) for the evolution and phylogenetics of the *Aeridinae* subtribe, Dwiati and Susanto (2021) for the intergeneric crosses between the genus *Phalaenopsis* and *Vanda*, and Jiang et al. (2021) for the chloroplasts genomic of the *Phalaenopsis zhejiangensis*.

Table 4 summarizes the genetic parameters for each of the five genera within the *Aeridinae* subtribe evaluated in this study. The estimated allele frequencies for presence (p) and absence (q) of the amplified PCR product ranged from 0.00-0.26 and 0.74-1.00 (Table 4), respectively. The number of alleles (Na) for each genus varied between 0.00-2.00, while the number of effective alleles (Ne) for each genus ranged from 1.00 to 1.62 (Table 4). The expected heterozygosity (He) for each genus ranged from 0.00 to 0.37, and the observed heterozygosity (Ho) for each genus ranged from 0.00 to 0.49 (Table 4).

This study showed that none of the evaluated SSR primers generated amplified products in both the *Vascostylis* and *Aerides* accessions since the frequency of band present (p)=0 (Table 4). Meanwhile, for *Vanda*, *Arachnis*, and *Aranthera*-the frequency of band presence ranged from 0.13 to 0.26 (Table 4). Such results showed that the tested SSR primers (17 SSR primers) were not informative for evaluating the *Vascostylis* and *Aerides* accessions. The failure to obtain PCR amplified DNA from both the *Vascostylis* and *Aerides* accessions using the evaluated primers maybe because the two genera are the most distantly related to other accessions in the *Aeridinae* subtribe (Peyachoknagul et al. 2014). Hence, the target flanking sequences for SSR primer annealing in the *Vascostylis* and *Aerides* are different from the other genera. These primers are unable to bind and generate PCR products.

Table 3. The estimated allele present (p) and absence (q) frequencies, samples size (N), Allele numbers (Na), effective alleles numbers (Ne), expected heterozygosity (He), observed heterozygosity (Ho), major allele frequency (MAF), allele diversity (D), and the polymorphic information content (PIC) for each SSR primers across 30 *Aeridinae* subtribe orchid accessions

Primers	p	q	N	Na	Ne	He	Ho	MAF	D	PIC
C32	0.68	0.32	30.00	6.00	6.60	0.98	1.20	0.77	0.33	0.27
C106	0.66	0.34	30.00	6.00	6.53	0.94	1.16	0.81	0.29	0.24
C208	0.67	0.33	30.00	6.00	6.57	0.97	1.18	0.78	0.31	0.25
C225	0.26	0.74	30.00	4.00	5.61	0.44	0.51	0.85	0.25	0.21
C268	0.71	0.29	30.00	6.00	6.64	0.99	1.20	0.79	0.30	0.25
C359	0.75	0.25	30.00	6.00	6.74	1.03	1.25	0.76	0.32	0.26
MOK26	0.59	0.41	30.00	6.00	6.40	0.91	1.11	0.80	0.30	0.25
MOK29	0.67	0.33	30.00	6.00	6.53	0.93	1.14	0.79	0.32	0.26
MOK62	0.83	0.17	30.00	6.00	6.92	1.10	1.34	0.82	0.28	0.24
MOK103	0.60	0.40	30.00	6.00	6.40	0.90	1.11	0.82	0.28	0.24
MOK107	0.76	0.24	30.00	6.00	6.67	1.01	1.22	0.80	0.29	0.24
VMJ05	0.25	0.75	30.00	2.00	5.57	0.34	0.35	0.68	0.40	0.31
VMJ08	0.55	0.45	30.00	6.00	6.29	0.85	1.05	0.86	0.24	0.21
VMJ09	0.61	0.39	30.00	6.00	6.43	0.94	1.14	0.78	0.34	0.28
VMJ10	0.62	0.38	30.00	6.00	6.46	0.92	1.13	0.82	0.28	0.24
VMJ14	0.74	0.26	30.00	6.00	6.71	1.01	1.24	0.81	0.29	0.24
VMJ31	0.65	0.35	30.00	6.00	6.51	0.93	1.15	0.81	0.28	0.23
Average	0.62	0.38	30.00	5.65	6.45	0.89	1.09	0.80	0.30	0.25

Tabel 4. The estimated allele present (p) and absence (q) frequencies, samples size (N), Allele numbers (Na), effective allele numbers (Ne), expected heterozygosity (He), and observed heterozygosity (Ho) across five *Aeridinae* subtribe orchid accessions across 17 SSR primers.

Genera	P	Q	N	Na	Ne	He	Ho
Vanda	0.13	0.87	23	2.00	1.28	0.20	0.20
Arachnis	0.24	0.76	3	1.88	1.54	0.33	0.40
Aranthera	0.26	0.74	2	1.76	1.62	0.37	0.49
Vascostylis	0.00	1.00	1	0.00	1.00	0.00	0.00
Aerides	0.00	1.00	1	0.00	1.00	0.00	0.00
Average	0.13	0.87	6	1.13	1.29	0.18	0.22

Sequence differences at the primer binding sites result in non-amplification (Suni 2017). Other explanations include new motifs in the genome sequences at the primer annealing site (Lee et al. 2020) and the heterozygous deficit phenomenon (Chen et al. 2014; Martel et al. 2020). Even though the 17 tested SSR primer loci were unable to generate any PCR product (SSR markers) for the *Vascostylis* and *Aerides*, they are informative in generating SSR markers for the accessions of *Vanda*, *Arachnis*, and *Aranthera* genera. Therefore, the tested SSR primers may be used as references for the molecular analysis of the *Vanda*, *Arachnis*, and *Aranthera* genera.

Genetic distance among *Aeridinae* accessions

Pairwise genetic distances among *Aeridinae* accessions based on evolutionary dissimilarities were calculated for single data using DARWin software (Perrier and Jacquemoud-Collet, 2014), and the results were presented in Table 5. Meanwhile, the frequencies of the pairwise dissimilarity values are presented in Figure 2. Most of the pairwise dissimilarity values among *Aeridinae* accessions fall between 0.7 to 0.9 (Figure 2).

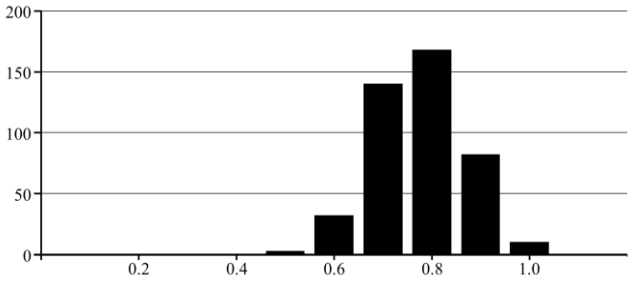

Figure 2. The frequencies of pairwise dissimilarity values among evaluated 30 *Aeridinae* accessions

Table 5. Pairwise dissimilarity matrix of 30 accessions of *Aeridinae* subtribe orchid collections calculated using the Weighted Neighbor-Joining method. The calculation was conducted using DARwin software version 6.0.21, and the genotype data were derived from 17 polymorphic SSR primers

	1*	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**
	ASDLP	**VsVQ1**	**Vi**	**VsAFs**	**VC**	**Vl**	**AS**	**VPR**	**AJS**	**ALG**	**AMO**	**Am**	**Vt**	**AFa**	**VUGDAF**	**ALPGCL**	**VDAt**	**VFDI**	**Ao**	**VAATV**	**VRDib**	**VRDp15**	**VPD**	**VMR**	**Vs**	**VES**	**AGCL114**	**VRDb**	**VGBM**
VsVQ1	0.68																												
Vi	0.64	0.65																											
VsAFs	0.66	0.58	0.64																										
VC	0.64	0.69	0.59	0.59																									
Vl	0.71	0.50	0.64	0.60	0.70																								
AS	0.81	0.85	0.83	0.81	0.83	0.86																							
VPR	0.71	0.73	0.73	0.65	0.63	0.68	0.93																						
AJS	0.81	0.80	0.79	0.82	0.83	0.78	0.77	0.77																					
ALG	0.72	0.64	0.78	0.65	0.79	0.72	0.68	0.78	**0.45**																				
AMO	0.66	0.73	0.75	0.70	0.80	0.82	0.64	0.82	0.57	0.51																			
Am	0.71	0.63	0.71	0.69	0.77	0.72	0.64	0.84	0.71	0.69	0.70																		
Vt	0.73	0.68	0.60	0.73	0.70	0.73	0.77	0.67	0.74	0.76	0.79	0.69																	
AFa	0.79	0.88	0.84	0.76	0.87	0.91	0.95	0.85	0.95	0.85	0.84	0.76	0.89																
VUGDAF	0.65	0.64	0.64	0.63	0.58	0.71	0.92	0.65	0.73	0.77	0.86	0.76	0.70	0.74															
ALPGCL	0.77	0.68	0.64	0.56	0.57	0.64	0.90	0.65	0.83	0.78	0.82	0.78	0.66	0.80	0.54														
VDAt	0.65	0.66	0.68	0.66	0.77	0.63	0.85	0.73	0.73	0.77	0.74	0.70	0.69	0.87	0.57	0.64													
VFDI	0.68	0.69	0.63	0.74	0.64	0.74	0.82	0.73	0.79	0.76	0.75	0.78	0.67	0.87	0.60	0.63	0.58												
Ao	0.81	0.88	0.85	0.77	0.85	0.85	0.81	0.89	0.69	0.77	0.81	0.85	0.77	0.85	0.78	0.78	0.81	0.85											
VAATV	0.62	0.66	0.71	0.69	0.69	0.77	0.89	0.73	0.76	0.78	0.79	0.71	0.70	0.88	0.64	0.59	0.62	0.70	0.84										
VRDib	0.67	0.72	0.73	0.73	0.62	0.70	0.81	0.71	0.76	0.76	0.77	0.79	0.68	0.88	0.67	0.66	0.65	0.69	0.79	0.64									
VRDp15	0.68	0.62	0.64	0.59	0.63	0.68	0.90	0.63	0.83	0.78	0.70	0.79	0.67	0.78	0.59	0.56	0.61	0.70	0.73	0.67	0.61								
VPD	0.69	0.54	0.64	0.73	0.63	0.68	0.84	0.56	0.82	0.77	0.78	0.68	0.73	0.80	0.67	0.69	0.65	0.66	0.90	0.69	0.67	0.66							
VMR	0.67	0.71	0.62	0.70	0.67	0.72	0.86	0.82	0.72	0.80	0.79	0.75	0.50	0.84	0.59	0.68	0.57	0.73	0.72	0.66	0.61	0.69	0.69						
Vs	0.66	0.57	0.66	0.66	0.65	0.63	**0.98**	0.73	0.85	0.83	0.81	0.67	0.71	0.78	0.66	0.62	0.59	0.74	0.81	0.60	0.67	0.71	0.63	0.65					
VES	0.70	0.65	0.65	0.70	0.60	0.73	0.76	0.72	0.61	0.59	0.64	0.70	0.73	0.84	0.71	0.72	0.67	0.72	0.77	0.72	0.68	0.63	0.65	0.75	0.67				
AGCL114	0.67	0.71	0.64	0.62	0.56	0.66	0.90	0.61	0.80	0.72	0.79	0.83	0.64	0.84	0.57	0.53	0.65	0.68	0.75	0.64	0.69	0.61	0.67	0.55	0.65	0.74			
VRDb	0.75	0.65	0.77	0.76	0.73	0.77	0.89	0.85	0.80	0.83	0.89	0.77	0.76	0.92	0.58	0.82	0.66	0.75	0.82	0.72	0.61	0.79	0.73	0.58	0.65	0.77	0.70		
VGBM	0.70	0.71	0.77	0.79	0.81	0.80	0.76	0.77	0.84	0.75	0.83	0.75	0.85	0.86	0.76	0.79	0.70	0.70	0.92	0.73	0.66	0.70	0.76	0.71	0.75	0.76	0.70	0.78	
VtAPF21	0.77	0.75	0.72	0.80	0.80	0.75	0.82	0.85	0.72	0.75	0.84	0.77	0.72	0.88	0.73	0.84	0.77	0.76	0.90	0.83	0.82	0.79	0.81	0.77	0.79	0.87	0.82	0.74	0.75

Note: *The numbers stand for the accession numbers, and their complete names are represented in Table 1

The calculated pairwise dissimilarity values among 30 *Aeridinae* accessions evaluated in this study ranged from 0.45 to 0.98 (Table 5). The highest pairwise dissimilarity value (0.98) was between *Armodurum sulingii* (As) and *Vanda sanderiana* (Vs) (Table 5). The smallest pairwise dissimilarity value (0.45) was between *Aranthera* James Storii (AJS) and *Aranthera* Ladda Gem (ALG) (Table 5), which belong to terete *Vanda* orchids having a starflower shape, open petal, and open sepal arrangement, speckled color pattern, brownish-red flower color. Both AJS and ALG grow well under direct sunlight (Wegadara et al. 2021). Hybridization between AJS and ALG is known to produce viable seedlings. Such observation aligns with earlier reports that accessions showing high similarity based on their genetic distance also share similar plant phenotypes (Kotilinek et al. 2020) and are cross-compatible (Hartati et al. 2017; Hartati et al. 2019).

The pairwise dissimilarities values show the pairwise genetic distances among evaluated *Aeridinae* accessions. Therefore, the pairwise dissimilarity values may be used to select potential male and female parent combinations in the hybridization schemes. A pair of samples showing high pairwise dissimilarity value showed they were distantly related, while those showing low pairwise dissimilarity value showed they were closely related. Hybridization among male and female parents that are distantly related should produce diverse offspring variability, and progenies having unique characters may be selected from the hybrid population. Closely related accession (intergeneric or interspecific) has been used to generate new orchid varieties with unique flowers (Hartati et al. 2019; Antonetti et al. 2021). However, intergeneric hybridizations are rarely successful (Kim et al. 2015; Hartati et al. 2019; Dwiati and Susanto 2021) because they are often sexually incompatible. The sexual incompatibility may be because of incompatible pollen and stigma shapes, pollen germination failure, or pollen tube growth failure (Kim et al. 2015). However, embryonic degeneration, improper embryo development or growth, and aberrant or partial endosperm development may also be the reasons (Kim et al. 2015).

Intergeneric cross-compatibility often occurs among members of the *Aeridinae* subtribe, thus complicating the biological concept of species in orchids (Surveswaran et al. 2018). New knowledge about the evolutionary process of the new species emergence has revised the concept of species (Aung and Jin 2018; Zhou et al. 2021). Constraints associated with reproduction, seed dispersal, germination, and habitat disturbance may affect gene flow and threaten certain species to extinction. Hence, those constraints affect the position of species in the phylogenetic tree (Minasiewicz et al. 2018; Yun et al. 2020). The discovery of new species from crosses also regularly changes the grouping and name of an orchid species (Kurniawati et al. 2019). The genetic distance calculated using molecular marker data may be used to predict the existence of sexual incompatibility among members of the *Aeridinae* subtribe.

Factorial analysis based on the SSR marker

Figure 3 presents principal component analysis (PCoA) results for 30 accessions of *Aeridinae* subtribes based on their amplified SSR marker profiles. The PCoA was calculated using the factorial analysis in the DARWin Software (Perrier and Jacquemoud-Collet 2014). The factorial analysis resulted in 25 principal components (PC), and the total 25 PCs explain the cumulative data variance of only 27.10%. The factorial analysis also showed that the 30 accessions from five genera of the *Aeridinae* subtribe were distinguished along axis 1, which explained 3.9% of the total variances. In contrast, axis 2 captured 2.3% and distinguished the *Aerides*, *Arachnis*, and *Aranthera* (full sun tolerance) from *Vanda* and *Vascostylis* (full sun-sensitive) genera (Figure 3).

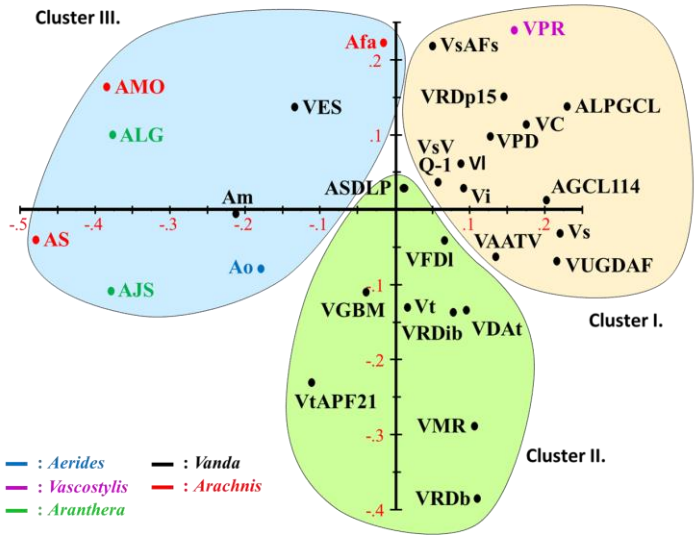

Figure 3. Principal component analysis (PCoA) of SSR data for the 30 accessions of five genera of the *Aeridinae* subtribe orchid genetic resource collection of Indonesian Ornamental Crops Research Institute (IOCRI). See Table 1 for complete names of the abbreviated accessions

Factorial analysis divided the 30 accessions into three main groups, as presented in Figure 3. Cluster I consisted of 13 accessions, Cluster II consisted of 9 accessions, and Cluster III consisted of 8 accessions (Figure 3). Based on PCoA analysis results, *Aeridinae* accessions belonging to Cluster I include AGCL114, ALPGCL, VAATV, VC, Vi, Vl, VPD, VPR, VRDp15, Vs. VsAFs, VsVQ-1, and VUGDAF (Figure 3, see Table 1 for complete accession names). Accessions belonging to Group II include ASDLP, VDAt, VFDI, VGBM, VMR, VRDb, VRDib, Vt, and VtAPF21 (Figure 3, see Table 1 for complete accession names). Meanwhile, accessions belonging to Group III include Afa, AJS, ALG, Am, AMO, Ao, AS, and VES (Figure 3, see Table 1 for complete accession names). The PCoA is often used as a multivariate statistical tool to explain native morphological, genetic, and biochemical variations within and between accessions, populations, and species (Vafaee et al. 2021). Fajardo et al. (2014) reported similar PCoA values for interspecific orchid accessions collected in Brazil.

Phylogenetic analysis of Subtribe *Aeridinae*

Phylogenetic analysis results for five genera of the *Aeridinae* subtribe, such as *Aranthera*, *Arachnis*, *Aerides*, *Vanda*, and *Vascostylis*, are presented in Figure 4. Thirty accessions of the evaluated *Aeridinae* subtribe were divided into three major clusters. Member of Cluster I (Figure 4) consisted of 13 accessions (Cluster I.a.-three, Cluster I.b.-four, and Cluster I.c.-six accessions). Cluster II consisted of 9 accessions (Cluster II.a.-six and Cluster II.b.-three accessions), and Cluster III consisted of eight accessions (Figure 4). Accessions belonging to Cluster I.a. include Vi, VMR, and Vt; Cluster I.b. include AGCL-114, ALPGCL, VC, and VUGDAF; and Cluster I.c. Include Vl, VPD, VPR, VRDp15, VsAFs, and VsVQ1 (Figure 4, see Table 1 for complete accession names). Accessions belonging to Cluster II.a. include VDAt, VFDI, VGBM, VRDb, VRDib, and VtAPF21; and Cluster II.b. include ASDLP, VAATV, and Vs (Figure 4, see Table 1 for complete accession names). Members of Cluster I.a., I.b., II.a., and II.b. all consisted of *Vanda* accessions, while those of Cluster I.c. consist of one *Vascostylis* accession and five *Vanda* accessions. Accessions belonging to Cluster III. include Afa, AJS, ALG, Am, AMO, Ao, AS, and VES (Figure 4, see Table 1 for complete accession names). In Cluster III, eight accessions from four different genera (two accessions of *Vanda*, two *Aranthera*, three *Arachnis*, and one *Aerides*) are grouped.

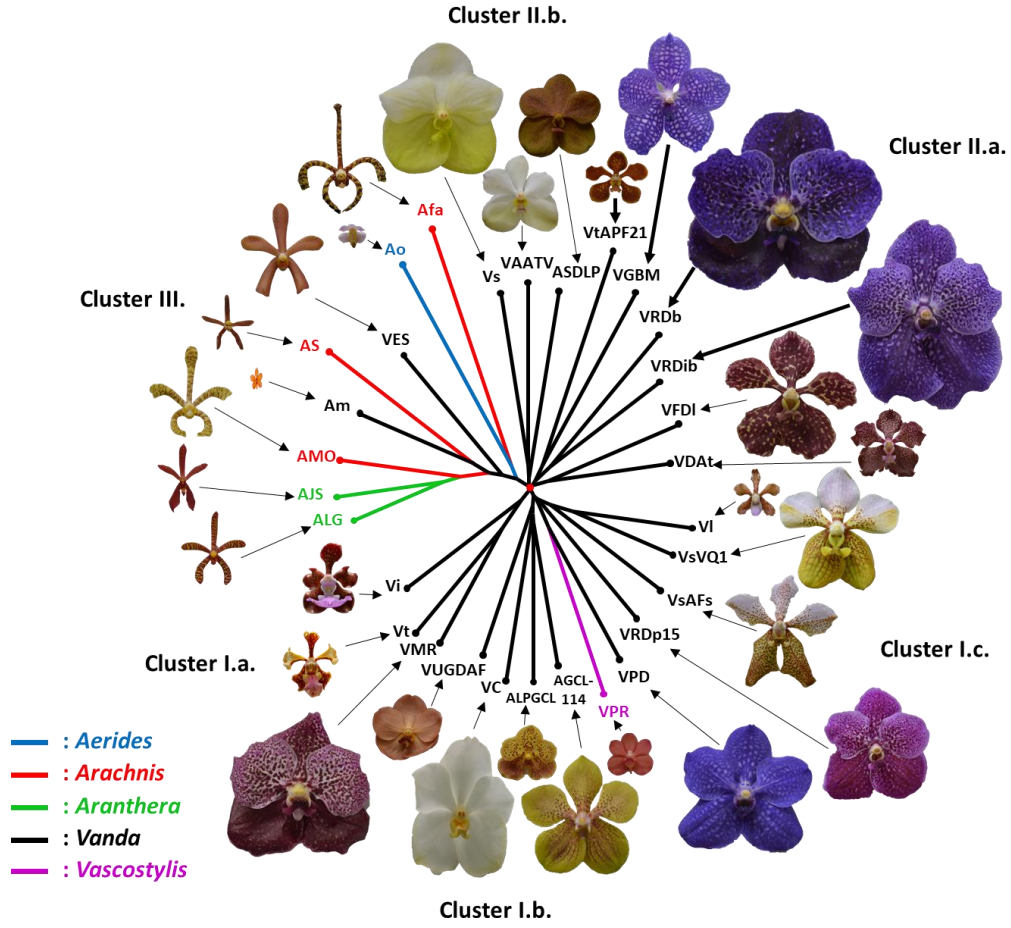

Figure 4. Dendrogram showing the phylogenetic relationship among 30 accessions belonging to five genera of the *Aeridinae* subtribe orchid of IOCRI genetic resource collection, based on the Weighted Neighbor-Joining method using DARwin software version 6.0.21 using genotype data derived from 17 polymorphic SSR markers

Factorial analysis and phylogenetic tree construction are two different approaches to present diversity structure. The factorial analysis (PCoA) gives an overall diversity representation, not individual effects. Meanwhile, phylogenetic tree construction focuses on individual sample relationships and less on the overall diversity structure. However, the two approaches should evaluate the data (Perrier and Jacquemoud-Collet 2014). Genetic diversity representation results are primarily similar in this study based on factorial analysis and phylogenetic tree construction. Clustering 30 accessions of *Aeridinae* subtribes using both approaches resulted in three main clusters (Figure 3 and Figure 4) and most of the clusters share similar accessions as their members. However, results of the factorial analysis clustered VAATV and Vs in Cluster I, and results of phylogenetic tree analysis clustered the two accessions as members of Cluster II.a. On the other hand, results of the factorial analysis clustered VMR and Vt in Cluster 2, and the phylogenetic analysis placed the two accessions as the member of Cluster I.a. Both factorial analysis and phylogenetic tree approaches placed the same eight accessions (Afa, AJS, ALG, Am, AMO, Ao, AS, and VES) in the Cluster III.

According to Hidayat et al. (2012), members of the *Aeridinae* subtribe are distinguished by two or four strong pollinia with well-developed stipe and viscidium, while column foot spurred lip distinguishes other genera. Clustering the *Aeridinia* subtribe accessions based on the SSR marker data in this study gave different results than those based on the morphological characters previously described by Wegadara et al. (2021). In a previous study, the *Vanda* and *Vascostylis* were grouped into one Cluster based on morphological data (Wegadara et al. 2022). In this study, the two genera are also grouped into one Cluster (Cluster I.c.) based on the SSR marker data. Furthermore, the *Aerides*, *Arachnis*, and *Aranthera* belong to one cluster based on either morphological (Wegadara et al. 2021) or SSR marker data (Cluster III, in this study). The diversity of orchids and their adaption to various environmental factors may contribute to phenotypic variability and eventually affect the grouping based on the morphological characters (Morales et al. 2010; Blinova 2012; Zhang et al. 2015; Dong et al. 2018; Evans et al. 2021). Such conditions may cause different clustering based on the morphological characters and the SSR data.

Molecular markers, such as SSRs, have been used to determine the genetic relationship among individuals within a species, among species, and among genera (Zhao et al. 2019; Lee et al. 2020; Kim et al. 2020; Yun et al. 2020). The markers have also been used to aid parental choice in hybridization to generate diverse progenies and support plant breeding activities (Lema 2018; Lamichhane and Thapa 2022). However, SSR markers' use for characterizing *Aeridinae* orchids is still needed, mainly since many species belong to this *Aeridinae* subtribe worldwide (Chase et al. 2015). This study evaluates phylogenetic relationships among 30 accessions belonging to five genera of the *Aeridinae* subtribe. This study's results are different from those of other studies (Givnish et al. 2015; Niu et al. 2017; Shi et al. 2018) because of differences in the accessions and the SSR loci evaluated. More studies are still needed to employ SSR markers for species differentiation, clone or variety identification, genetic linkage map, and marker-assisted selection in the *Aeridinae* subtribe. Marker-assisted selection for various flower characters should benefit the *Aeridinae* subtribe because these orchids have a long juvenile (vegetative) period (Kartikaningrum et al. 2015). Their flowers are the desired characters. Therefore, identifying SSR markers associated with flower characters should be the aim of future research.

Hybridization among closely related accessions to generate intergeneric or interspecific hybrids may create new varieties with unique flower types (Hartati et al. 2019; Antonetti et al. 2021) and generate more diversities. However, intergeneric hybrids are usually more difficult to generate (Kim et al. 2015; Hartati et al. 2019; Dwiati and Susanto 2021) because of pollen shape and stigma incompatibility, pollen germination, and pollen tube growth failure (Kim et al. 2015). Moreover, embryo degeneration, abnormal embryo development or growth, and aberrant or partial endosperm development may also cause the failure to generate intergeneric hybrids (Kim et al. 2015).

This study showed that interspecific hybrids among accessions of *Vanda* within Cluster I and Cluster II (Figure 4) should be possible since they are genetically closely related. Moreover, intergeneric hybrids among the accession of *Vascostylis* (VPR) and *Vanda* genera belonging to Cluster I.c. (Vl, VPD, VRDp15, VsAFs, and VsVQ1) should also be possible because of the close genetic relationship. Furthermore, intergeneric hybrids among *Aerides* (AO), *Arachnis* (Afa, AMO, and AS), *Aranthera* (AJS and ALG), and *Vanda* (Am and VES) accessions within Cluster III should also be possible based on their close genetic distance (Figure 4).

The *Aeridinae* subtribe is part of the subfamily Epidendroideae, having diverse phenotypic variations and generating intergeneric hybrids among genera within the *Aeridinae* subtribe. The presence of intergeneric hybrids within the *Aeridinae* subtribe has contributed to the complexity of the biological concept of species in orchids (Surveswaran et al. 2018) and eventually revised the modern species concept (Aung and Jin 2018; Zhou et al. 2021). The new species discovery from intergeneric and interspecific hybrids results in regular changes in the grouping and naming of orchid species (Kurniawati et al. 2019).

In conclusion, molecular characterization of 30 orchid subtribes *Aeridinae* genetic resources of IOCRI was carried out using 17 SSR markers. When evaluated using QIAxcel, the 17 SSR primers generated 240 amplified DNA fragments across 30 accessions, with the fragment sizes ranging from 56-4818 bp. Factorial analysis (PCoA) and phylogenetic tree construction clustered the 30 *Aeridinae* accessions into three major groups. Accessions belonging to the *Vanda* genus are clustered into three clusters (Clusters I, II, and III). The *Vaschostylis* is in Cluster I, along with 12 with *Vanda* accessions. Two accessions of *Vanda*, two *Aranthera*, three *Arachnis*, and one *Aerides* genera are clustered into Cluster III. This study showed that

interspecific hybrids among accessions of *Vanda* within Cluster I and Cluster II should be possible since they are genetically closely related. Intergeneric hybrids among the accession of *Vascostylis* and *Vanda*'s genera belonging to Cluster I. should also be possible. Furthermore, intergeneric hybrids among *Aerides*, *Arachnis*, *Aranthera*, and *Vanda* accessions within Cluster III should also be possible. The generated data from this study should be helpful for future *Aeridinae* breeding activities intended to generate new hybrid varieties.

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