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Characteristics of DNA Barcodes from Three *Thrixspermum* Orchids Based on *ITS2* Regions

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Abstract. *Thrixspermum (T.)* is one of the genus in Orchidaceae that has small flowers. Among species in this genus has a high homology and also has many synonyms. Identification using morphological characters can be constrained since *Thrixspermum* flowering time occurs in a very short period. This study aimed to conduct molecular-based identification of *Thrixspermum* orchids using DNA barcoding. This method applied molecular-based species identification technique using DNA sequences from genomic fragments that are considered fast, accurate, and consistent. The molecular markers used were *Internal Transcribed Spacer 2 (ITS2),* while the samples used were *T. centipeda, T. lucidum,* and *T. angustifolium.* BLAST results show that *T. centipeda* has a close relationship with *T. centipeda* from Malaysia (KX679332) with 99.79% percent identity, *T. lucidum* has a high homology with *T. linusii* (KX679333; 97.30%), while *T. angustifolium* has a high homology with *T. triangulare* (KX679348; 99.38%). There is a unique sequence that only *T. lucidum* and *T. angustifolium* have that distinguishes the two from other species. *ITS2* can be recommended as a molecular marker for determining the *Thrixspermum* orchid barcode. The benefit obtained from this research is the DNA barcode sequences (*ITS2*) of *Thrixspermum* orchids would be very useful to enrich the plant barcodes database for further molecular taxonomy and biodiversity of orchid.

Key words: DNA barcode*; ITS2* fragment*;* molecular marker; *Thrixspermum*

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INTRODUCTION

Orchids are diverse group of flowering plants with up to 26,000 species belonging to 760 genera (Goldblatt & Manning, 2019) and 6,000 species of which are found in Indonesia (Fandani et al., 2018). The habitat of orchid is widespread from the lowlands to the mountains and from the wet forest to the dry forest. Most types of orchids belong to the CITES Appendix II category (Sadili, 2011). Forest damage will threaten the existence of orchids (species) that live epiphytically in the forest trees, one of which is the *Thrixspermum* orchid.

Thrixspermum (*T.*) is a genus of members of Orchidaceae which is spread in tropical and subtropical regions from Asia to Australia and the Pacific (O'Byrne, 2016). Its lives as an epiphyte by attaching to tree trunks (Nurfadilah, 2015). *Thrixspermum* orchids have small flowers and have the characteristic of flowering in a short time. The morphological characters of *Thrixspermum* leaves and flowers are the basis of phenotypic markers to distinguish this plant group (Wulanesa et al., 2017). The species belong to this genus has a similar morphology with its closest relatives, for example *T. tsii* which has a vegetative characteristic similar to *T. centipeda,* but the flower structure is similar to *T. subulatum* (Chen & Shui, 2005).

Thrixspermum orchids have often been ignored and rarely cultivated because they are less in demand. Therefore, research reports about characterization and potential of *Thrixspermum* orchids is limited. In addition, local or several different names also confuse people or researchers to give names for these species. This condition bring about obstacles in identification based on morphological characters (Rohimah et al., 2018), so an alternative identification based on molecular characters, what so called DNA barcoding, is indispensable.

DNA barcoding is a molecular-based species identification technique using DNA sequences from genomic fragment that are considered fast, accurate, and consistent, so it is important in the study of conservation biology and species diversity. For example, the method has been applied to explore the biodiversity of fungi, insects (thrips), birds, and fish (Kurniawaty et al., 2016; Ashari & Astuti, 2017; Hasanah et al., 2017; Nuryanto et al., 2018a; Nuryanto et al., 2018b). This method is based on the concept that each species has a unique genetic identity. Identification with DNA sequences is carried out using molecular markers. The molecular markers used in expressing taxon-

omies are short sequences of DNA that can show genetic variation among individual species or organisms (Idrees & Irshad, 2014).

The process of species identification in the *Thrixspermum* orchid begins with genomic DNA isolation and PCR amplification. The primer used was *Internal Transcribed Spacer 2* (*ITS2*) derived from nucleus DNA, which is one of the most commonly used molecular markers in phylogenetic analysis and DNA barcoding of plants and has been recommended as a barcode of plant core DNA (Cheng et al., 2016). *ITS2* sequence has been used as a strong phylogenetic marker at the species level and showing a high degree of interspecific difference (Hollingsworth et al., 2011). Therefore this study was conducted to identify potential short sequences as specific barcodes on *Thrixspermum* orchids using *ITS2* molecular markers. This is the first report for the exploration of *Thrixspermum* orchids with a molecular approach. Therefore, the sequence data (*ITS2*) originated from three *Thrixspermum* species used in this study is indispensable for supporting the subsequent research, especially for molecular taxonomy and phylogenetic analysis of orchid.

METHODS

Genomic DNA Isolation

Genomic DNA of orchid samples (*Thrixspermum centipeda*, *Thrixspermum lucidum*, *Thrixspermum angustifolium*) were isolated using Cetyltrimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1985) with minor modification. Briefly, about 0.5 g samples were pulverized with mortar and pestle and homogenized with CTAB buffer containing RNAse. The homogenate was then incubated at 65°C chamber for 1 hour, then added with 500 µl chloroform and incubated at room temperature for 5 minutes. After that, the homogenate was centrifuge at 10.000 rpm, 4°C for 15 minutes. The next processes were performed following the manufacture's instruction (Nexprep DNA Kit, Korea). The genomic DNA from each sample was then used as a template for PCR amplification.

PCR Analysis and Electrophoresis

PCR reaction mixture consists of 1.5 µl of template DNA, 1 µl of both forward and reverse primers (DR2F, 5'-GGCTCTCGCATCGATGAAGA-3' and *ITS*_26SE, 5'-TAGAATTCCCCGGTTCGCTCGCC GTTAC-3'), 10 µl 2×Go Taq® Green PCR Master Mix (Promega, USA), and deionized distilled water until total volume of 20 µl. The PCR condition was adjusted as: pre-denaturation at 95ºC for 5 min, followed by 35 cycles of denaturation at 95ºC for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min 15 sec, then final extension at 72°C for 5 min and hold at 16ºC. The amplified PCR products were loaded on 1.25% agarose gel containing ethidium bromide to confirm the presence or absence of bands. The electrophoresis machine was set at 100 Volt for 45 min, and then the PCR product was visualized on UV illuminator. The size of amplified PCR products was determined using NEXmark™ 100bp ladder. The PCR product was purified and the sequences were determined by employing sequencing service provided by Macrogen, Korea.

Sequence Analysis

Sequence analysis was conducted by using some bioinformatics software. The homology and alignment of each *ITS2* sequence from *Thrixspermum* samples were analyzed using Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI) and Clustal X (Chenna et al., 2003). The phylogenetic tree was constructed with MEGA7 software (Tamura et al., 2011).

RESULTS AND DISCUSSION

The three *Thrixspermum* orchids studied were included as monopodial orchids and didn't have the pseudobulb. The stems of *T. centipeda* are flat and the flowers are classified as a compound flower (*anthotaxis/inflorescentia*). The labellum has brown spots on newly bloomed flowers and red on wilted flowers with white patches on the tip (Figure 1) (Purnama et al., 2016). *T. centipeda* grows near river on tree branches (Purnama et al., 2016). This orchid was distributed in several regions, including China, Thailand, Vietnam, Cambodia, Malacca Peninsula, Sumatra, Java, Borneo, Philippines, and Laos (Averyanov et al., 2016). *T. centipeda* has several different names, such as *T. arachnites*, *T. papillosum*, and *Sarcochilus arachnites* (Rohimah et al., 2018). This orchid also has medicinal potential in Thailand to treat asthma by utilizing all parts of the plant (Teoh, 2016; Rohimah et al., 2018). *T. lucidum* has herbal habitus and green leaves with a flat leaf edge (*integer*). Flower of this orchid is small with turbid white sepal and petal, and white labellum. *T. angustifolium* has thick and fleshy leaves. It has green leaf color with a pointed tip (*acuminatus*) and a flat edge (*integer*). These orchid leaves have a smooth surface (*laevis*) (Figure 1).

DNA isolation is the first step in DNA analysis that serves to separate DNA from other materials such as polysaccharides, proteins, and fats. The isolated DNA using the modified CTAB method is used as a template in the amplification process. The process of DNA amplification using a Polymerase Chain Reaction (PCR) machine was carried out for 35 cycles. According to Herman et al. (2018), PCR is gen-

erally carried out between 25-40 cycles. PCR cycles with more than 35 will not significantly increase the number of amplicons and will allow an increase in the number of non-target products. The successful of

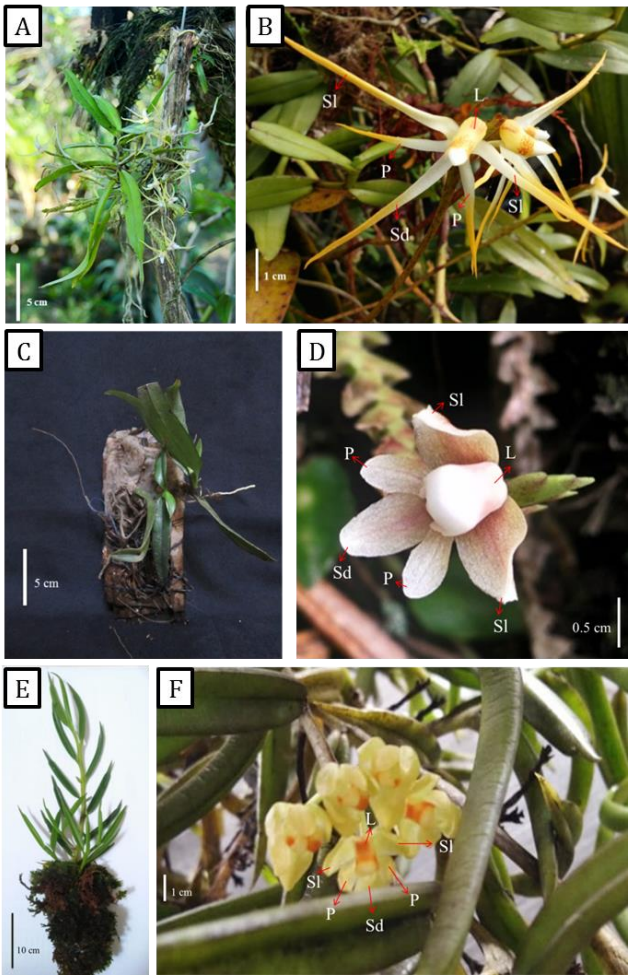

Figure 1. Vegetative morphology of *T. centipeda* (A), *T. lucidum* (C), *T. angustifolium* (E) and flower of *T. centipeda* (B), *T. lucidum* (D), *T. angustifolium* (F), note: dorsal sepal (Sd), lateral sepal (Sl), petal (P), and labellum (L)

PCR-based amplification used *ITS2* sequences on three species (*T. centipeda*, *T. lucidum*, and *T. angustifolium)* showing a clear and single band (Figure 2). This result showed that the DNA obtained was intact and perfectly amplified, and in addition, there was no smear band that was observed during amplification process. Visualization results also showed a single band of DNA that was thick and clear. This shows that the annealing temperature has been optimal to stick to the DNA template (Pangestika et al., 2015), so that DNA with good quality was obtained.

As shown in Figure 2, the DNA bands obtained from PCR amplification were between 400 to 500 bp in size. According to Takamiya et al. (2011), the size of DNA fragments amplified using DR2F and *ITS*_26SE primers is approximately 500 bp. The PCR DNA amplification is likely affected by the quality of DNA template, appropriate primer selection, and PCR condition.

product was then sequenced and analyzed. Sequencing is the stage of reading the sequence of DNA bases in amplified fragments (Sunaryo, 2015). Sequencing results obtained indicate that the sample can be sequenced well. According to Bangol et al. (2014), a perfect sequencing result can be observed by a graph with high peaks that are separated from each other.

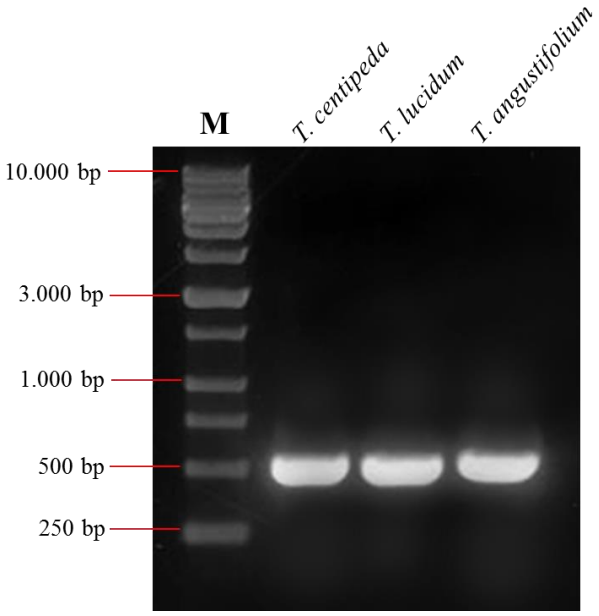

Figure 2. PCR product amplification of *ITS2* regions

Homology sequences of amplified sequences are detected using the Basic Local Alignment Search Tool (BLAST). Analysis using BLAST aims to find and analyze the homology level of an organism's sequence (Wardani et al., 2017). The alignment results of *Thrixspermum* orchids with *ITS2* primers (DR2F and 26SE) showed that *T. centipeda*, *T. lucidum*, and *T. angustifolium* had query length of 485 bp, 481 bp, and 482 bp, respectively. Further sequence analysis showed that *ITS2* primers were able to differentiate *Thrixspermum* orchids up to species level (Table 1).

The BLAST analysis was carried out by looking at score parameters of more than 150 and E-values of less than 10^{-4} or near zero (0), this shows that the homology level produced was quite good (Isda & Chaidamsari, 2013). The better homology level of an organism is characterized by the higher score (bits) and the lower E-value. Based on the BLAST analysis, zero (0) E-value showed that the alignment of all sequences was of significant value. The lower E-value indicates the higher homology between sequences, while the higher E-value indicates the lower homology between sequences (Fathiya et al., 2018).

The results of BLAST analysis showed that *T. centipeda* had the highest homology level with *T. centipeda* species originating from Malaysia (KX679332) with percent identity (% Ident) of 99.79%. Distinguishing sequence of both accessions is located in nitrogen base 324, namely Guanine (G) base in the *T. centipeda* sample which is different from the *T. centipeda* from Malaysia (GenBank) with the Adenine (A) base (Figure 3). Another difference between two sequences lies in the base sequence 495, namely Adenine (A) in the *T. centipeda* sample and Cytosine (C) bases owned by *T. centipeda* from Malaysia (GenBank). BLAST results also showed that *T. centipeda* has high homology with other types of *Thrixspermum*, namely *T. tortum* (KX679347) with percent identity of 99.79%, *T. caudatum* from Malaysia (KX679331) with 99.38%, *T. elongatum* (DQ091674) from USA with 99.75%, and *T. amplexicaule* and *T. tsii* from China with 99.48%.

Table 1. BLAST analysis of *ITS2* sequence on *T. centipeda*, *T. lucidum*, and *T. angustifolium*

T. centipeda				*T. lucidum*				*T.agustifolium*			
Name	Accession Number	% Ident	Source	Name	Accession Number	% Ident	Source	Name	Accession Number	% Ident	Source
T. centipeda	KX679332	99.79	MY	*T. linusii*	KX679333	97.30	MY	*T. triangulare*	KX679348	99.38	MY
T. tortum	KX679347	99.79	MY	*T. triangulare*	KX679348	96.47	MY	*T. tortum*	KX679347	98.54	MY
T. elongatum	DQ091674	99.75	USA	*T. caudatum*	KX679331	95.44	MY	*T. centipeda*	KX679332	98.54	MY
T. amplexicaule	KF545882	99.48	CN	*T. japonicum*	MK317966	95.44	KR	*T. caudatum*	KX679331	98.54	MY
T. tsii	KJ733459	99.48	CN	*T. tortum*	KX679347	95.23	MY	*T. tsii*	KJ733459	98.43	CN
T. caudatum	KX679331	99.38	MY	*T. centipeda*	KX679332	95.23	MY	*T. elongatum*	DQ091674	98.25	USA

Note : Malaysia (MY), United States of America (USA), China (CN), Korea (KR)

ITS2 sequence of *T. lucidum* showed the highest homology level with the *T. linusii* species from Malaysia with percent identity of 97.30%. There are 5 different base sequences in these two species, located in 411 base sequence between Thymine (T) and Adenine (A), base sequence 420 between Adenine (A) and Cytosine (C), base sequence 470 between Cytosine (C) and Thymine (T), base sequence 476 between Thymine (T) and Guanine (G), and base sequence 641 between Cytosine (C) and Thymine (T). Both of them also have the same bases at sequence 475 with Guanine (G), 555 with Thymine (T) and sequence 567 with the base of Cytosine (C) which are different from the other species. The alignment results of the *T. lucidum* alignment of the *ITS2* sequences also showed that there were 2 bases that distinguished *T. lucidum* from other species, located in base sequence 486 with the Cytosine (C) base and 689 with Thymine (T) base. In base sequence 687, *T. lucidum* sequence shows a gap that represents an insertion or deletion. Besides with *T. linusii*, *T. lucidum* also has a high homology with other types of *Thrixspermum*, namely *T. triangulare* (KX679348) and *T. caudatum* (KX679331) with percent identity of 96.47% and 95.44% respectively, and *T. tortum* (KX679332) and *T. centipeda* (KX679332) with 95.23%, those four accessions are from Malaysia. *T. lucidum* also has homology with *T. japonicum* (MK317966) from Korea with percent identity of 95.44%.

T. angustifolum ITS2 sequence has the highest homology level with *T. triangulare* (KX679348) from Malaysia with percent identity of 99.38%. Both of them have differences in the base sequence 324, namely Guanine (G) base owned by *T. angustifolium* and the Adenine (A) base owned by *T. triangulare*. Alignment results using *ITS2* sequences show that *T. angustifolium* has a base that is different from other species, namely Adenine (A) base located in sequence 477. BLAST results show that *T. angustifolium* also has high homology with *T. tortum* (KX679347), *T. centipeda* (KX679332), *T. caudatum* (KX679331) from Malaysia with per. Ident 98.54%, *T. tsii* (KJ733459) from China with per. Ident 98.43%, and *T. elongatum* (DQ091674) from USA with per. Ident 98.25%. The alignment results using the *ITS2* sequence show that in base sequence 324 bp, *T. centipeda* and *T. angustifolium* have the same base sequence, Guanine (G) which is not shared by other species. *ITS2* sequences have genetic variation and can differentiate to species level, so that *ITS2* has the

potential as a molecular marker (barcode) of the *Thrixspermum* orchid.

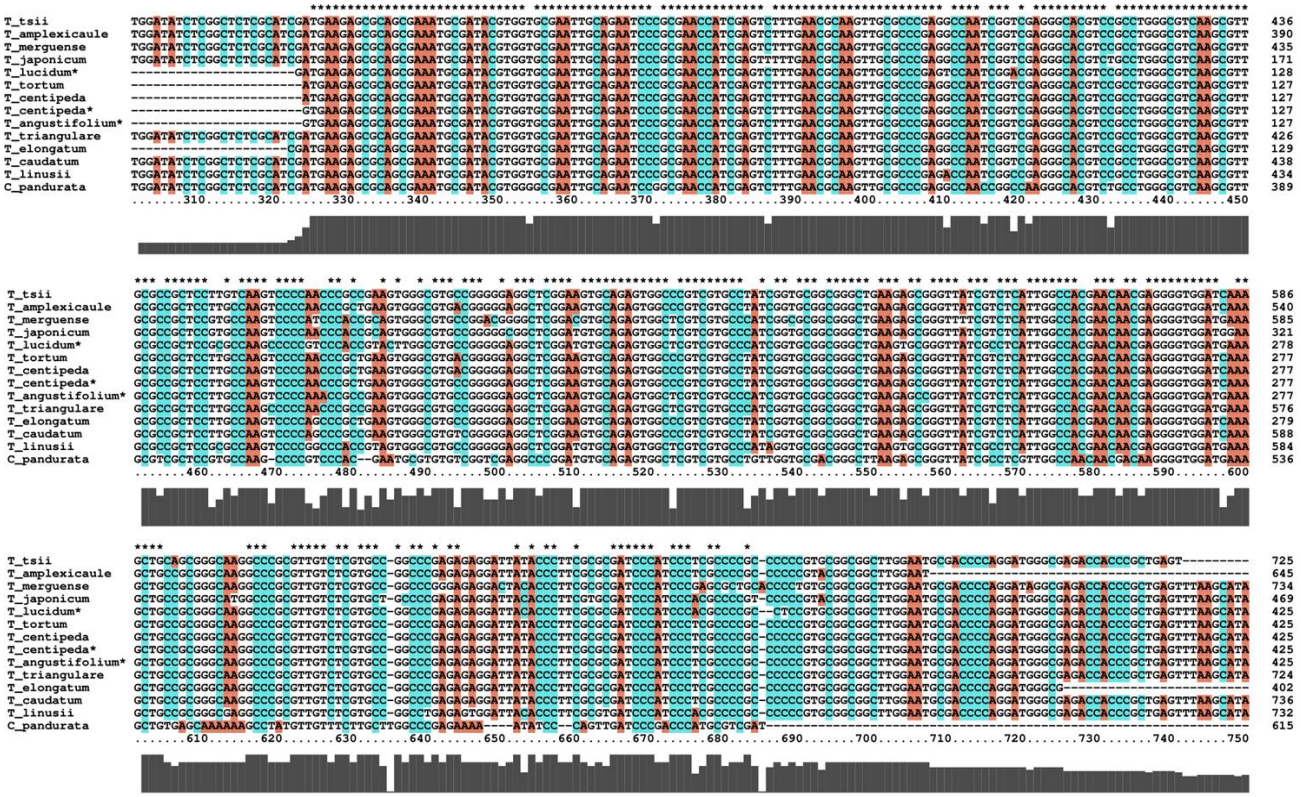

Figure 3. Alignment results of *ITS2* sequence from *T. centipeda*, *T. lucidum*, and *T. angustifolium* with closest relative in NCBI

Phylogenetic analysis was performed using MEGA 7 with the Neighbor-Joining (NJ) method which aims to reconstruct phylogenetic trees based on data in minimum evolutionary distances or the nearest ancestors (Li, 2015). Species that have high genetic homology would be on adjacent position in the phylogenetic tree. The results of phylogenetic reconstruction showed that the species of the genus *Thrixspermum* have the same ancestor (Figure 4). *T. angustifolium* is closely related to *T. triangulare*. Both of them are form one cluster and this is following the BLAST results that have been obtained. *T. lucidum* has a close genetic relationship with *T. linusii*. Both are thought to be oldest species of the genus *Thrixspermum*. In this phylogenetic analysis, *ITS2* sequence of *Coelogyne pandurata* is included as out-group. As seen in the figure, out-group are clearly separated from all *Thrixpermum* species since it has different characters genetical- and morphologically.

This study was expected to enhance sequence data collection in the DNA database or GenBank and also makes it easier for scientists to obtain *Thrixspermum* DNA sequences based on the *ITS2*. These sequences can be used to confirm the orchid species of *T. centipeda*, *T. lucidum*, and *T. angustifolium* and serve as the basis for further DNA barcoding studies.

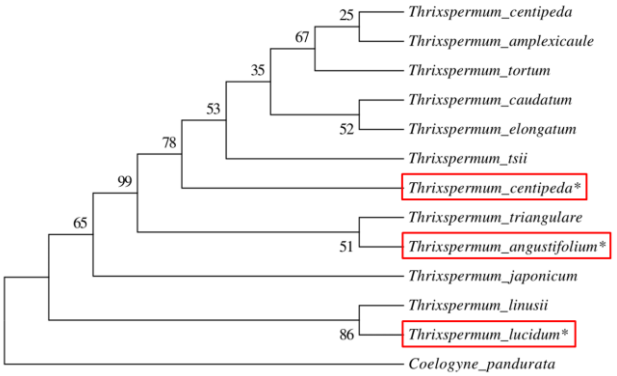

Figure 4. Phylogenetic tree of *ITS2* sequence *T. centipeda*, *T. lucidum*, and *T. angustifolium* with the closest relatives at NCBI

CONCLUSION

ITS2 sequences of *Thrixspermum* orchids have unique characteristic and was able to differentiate to the species level. Genetic relationship shows that the *Thrixspermum* orchids are originated from the same ancestor. Based on these results, *ITS2* sequences are highly recommended as a molecular marker to determine the barcode of the *Thrixspermum* orchids.

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REFERENCES

Ashari, H. & Astuti, D. (2017). Study on Phylogenetic Status of Javan Plover Bird (Charadrius, Charadriidae, Charadriiformes) through DNA Barcoding Analysis. *Biosaintifika: Journal of Biology & Biology Education, 9*(1), 49-57.

Averyanov, L. V., Nguyen, K. S., Maisak, T. V., Konstantinov, E. L., Nguyen, T. H. & Bounphanmy, S. (2016). New and Rare Orchids (Orchidaceae) in The Flora of Cambodia and Laos. *Turczaninowia, 19*(3), 5-58.

Bangol, I., Momuat, L. I., & Kumaunang, M. (2014). Barcode DNA Tumbuhan Pangi (*Pangium edule* R.) Berdasarkan Gen *matK*. *Jurnal Mipa UNSRAT Online, 3*(2), 113-119.

Chen, W. H., & Shui, Y. M. (2005). A New Species of *Thrixspermum* (*Orchidaceae*) from China. *Brittonia, 57*, 55-58.

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., & Zhou, S. (2016). Barcoding The Kingdom Plantae: New PCR Primers For ITS Regions Of Plants With Improved Universality And Specificity. *Molecular Ecology Resources, 16*, 138-149.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G., & Thompson, J. D. (2003). Multiple Sequence *Alignment* with the Clustal Series of programs. *Nucleic Acids Research, 31*(13), 3497-3500.

Doyle, J. J. & Doyle, J. L. (1985). Isolation of Plant DNA from Fresh Tissue. *L. H. Bailey Hortorium 466 Mann Library Building Cornell University Ithaca, New York.*

Fandani, H. S., Mallomasang, S. N., & Korja, I. N. 2018). Keanekaragaman Jenis Anggrek pada Beberapa Penangkaran di Desa Ampera dan Desa Karunia Kecamatan Palolo Kabupaten Sigi. *Jurnal Warta Rimba, 6*(3), 14-20.

Fathiya, N., Harnelly, E., Thomy, E., & Iqbar. (2018). Molecular Identification of Shorea johorensis In Ketambe Research Station, Gunung Leuser National Park. *Jurnal Natural, 18*(2), 56-64.

Goldblatt, P. & Manning, J. C. (2019). *Temperate Garden Plant Families: The Essential Guide to Identification & Calssification.* China: Timber Press, Inc.

Hasanah, U., Riwayati, Idramsa, Prasetya, E. (2017). Molecular Identification of Endophytic Fungi from Bark of Raru (*Cotylelobium melanoxylon*) that Produce the Antibacterial Compounds. *Biosaintifika: Journal of Biology & Biology Education, 9*(3), 380-386.

Herman, Nainggolan, M., & Roslim, D. I. (2018). Optimasi Suhu Annealing untuk Empat Primer RAPD pada Kacang Hijau (*Vigna radiata* L.). *Jurnal Dinamika Pertanian, 34*(1), 41-46.

Hollingsworth, P. M., Graham, S. W., & Little, D. P. (2011). Choosing and Using a Plant DNA *Barcode*. *PLoS One, 6*, Article e19254.

Idrees, M., & Irshad, M. (2014). Molecular Markers in Plants for Analysis of Genetic Diversity: A Review. *European Academic Research, 2*(1), 1513-1540.

Isda, M. N., & Chaidamsari, T. (2013). Analisis Sekuen Gen Proteinase Inhibitor (TcPIN) Terkait dengan Ketahanan Terhadap Penggerek Buah Kakao. *Prosiding Semirata FMIPA Universitas Lampung*.

Kurniawaty, N., Hidayat, P., & Rauf, A. (2016). Characterization of Three Species of Thrips on Banyan, Nutmeg, and Marine Seruni Plants Based on *COI* Gene. *Biosaintifika: Journal of Biology & Biology Education, 8*(2), 185-192.

Li, J. F. (2015). A Fast Neighbor Joining Method. *Genetics and Molecular Research, 14*(3), 8733-8743.

Nurfadilah, S. (2015). Diversity of Epiphytic Orchids and Host Trees (Phorophytes) In Secondary Forest of Coban Trisula, Malang Regency, East Java, Indonesia. *Biotropia, 22*(2), 120-128.

Nuryanto, A., Amalia, G., Khairani, D., Pramono, H., & Bhagawati, D. (2018b). Molecular characterization of four giant gourami strains from Java and Sumatra. *Biodiversitas, 19*(2), 578-584.

Nuryanto, A., Pramono, H., Kusbiyanto, Ghifari, M, I., & Andareswari. (2018a). Barcoding of Fin Clip Samples Revealed High Exploitation of *Plectropomus leopardus* in Spermonde Archipelago. *Biosaintifika: Journal of Biology & Biology Education, 10*(3), 629-635.

O'Byrne, P. (2016). *Trhixspermum* in Borneo: 24 New and Old Spesies. *Malesian Orchid Journal, 18*, 5-74.

Pangestika, Y., Budiharjo, A., Pancasakti, H., & Kusumaningrum. (2015). Analisis Filogenetik *Curcuma zedoaria* (Temu Putih) Berdasarkan *Gen Internal Transcribed Spacer (ITS)*. *Jurnal Biologi. 4*(4): 8-13.

Purnama, I., Wardoyo, E. R. P., & Linda, R. (2016). Jenis-jenis Anggrek Epifit di Hutan Bukit Luncit Kecamatan Anjongan Kabupaten Mempawah. *Protobiont, 5*(3), 1-10.

Rohimah, S., Mukarramah, L., Sindiya, V., Saputri, V. Y., Khoirunnisa, G. A. & Su'udi, M. (2018). Eksplorasi Jenis Dan Potensi DNA *Barcode* Ang-

grek *Thrixspermum* Secara *In Silico*. *Jurnal Biodjati, 3*(2), 148-156.

Sadili, A. (2011). Keanekaragaman, Persebaran dan Pemanfaatan Jenis-Jenis Anggrek (Orchidaceae) di Resort Citorek, Taman Nasional Gunung Halimun-Salak, Jawa Barat. *Biosfera*, *28*(1), 15-22.

Sunaryo, W. (2015). Aplikasi DNA Barcoding untuk Analisis Keragaman Genetik Lai-Durian (Durio zibethinus x kutejensis) Asal Kalimantan Timur. *Pros Sem Nas Masy Biodiv Indon*, *1*(6), 1273-1277.

Takamiya, T., Wongsawad, P., Tajima, N., Shioda, N., Lu, J. F., Wen, C. L., Wu, J. B., Handa, T., Ijima, H., Kitanaka, S., & Yukawa, T. (2011). Identification of Dendrobium Species Used for Herbal Medicines Based on Ribosomal DNA *Internal Transcribed Spacer Sequence*. *Biol. Pharm. Bull*, *34*(5), 779-782.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol*, *28*(10), 2731-2739.

Teoh, E. S. (2016). *Medicinal Orchids of Asia*. Singapura: Elsevier.

Wardani, M. T., Kusdiyantini, E., & Budiharjo, A. (2017). Identifikasi Isolat *Monascus* sp. Hasil Isolasi Angkak berdasarkan Gen *Internal Transcribed Spacer* (*ITS*) dan Pengukuran Kandungan Pigmen. *Jurnal Biologi 6*(2), 34-40.

Wulanesa, W. O. S., Soegianto, A., & Basuki, N. (2017). Eksplorasi dan Karakterisasi Anggrek Epifit di Hutan Coban Trisula Kawasan Taman Nasional Bromo Tengger Semeru. *Jurnal Produksi Tanaman*, *5*(1), 125-131.