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DNA Barcode of *Homalomena pexa* inferred from Internal Transcribed Spacer Region

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ABSTRACT

Homalomena pexa, a tomentose haired-leaf aroid, was a newly discovered and described plant species in early 2020. This species has currently only been reported from South Tapanuli. A molecular study to provide DNA sequence is essential in this preliminary investigation. This research aimed to characterize the barcode sequence of H. pexa and estimate the species' position in an Araceae phylogenetic tree. This research used ITS sequence to perform DNA barcoding on *H. pexa*. The sequencing result revealed that 1040 bp nucleotides were effectively amplified. The phylogenetic tree generated using the Neighbor-Joining method and the Kimura 2-parameter revealed that *H. pexa* clustered with *H. atrox* and *H. humilis*, with a bootstrap value of 97%. This investigation provided and demonstrated that ITS sequences could be used to validate and support the proper identification of Araceae species.

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INTRODUCTION

Homalomena Schott is a genus in the Araceae family, having a fairly wide distribution in tropical and subtropical regions, comprising 145 species (POWO, 2022). This genus is widely recognized as one of the most popular attractive leaf plants (Chen et al., 2007). Wong et al. (2020) described a new species, *Homalomena pexa*, with a moderately unusual morphological feature: all parts of the leaves are covered with tomentose trichomes. Previously, a coarsely ciliate trichomes on the leaf surface were only reported in *H. hasei* (Boyce and Yeng, 2016). In contrast to most *Homalomena* species, which have glossy leaf surfaces.

The published information about *H. bapexa* covers morphological and habitat characteristics. However, the specific location of *H. pexa* is concealed for the plant's conservation. Concealed information also means limited access to the plant's resources. Widiyanti and Mukarlina (2017) reported that forest fires and land conversion significantly impact Araceae's survival in the wild. Because the reported plant was not found in many other areas, genetic conservation of novel species that have not been thoroughly investigated is required to provide genetic information.

The sequence of DNA barcodes is frequently used to determine the species level using molecular techniques in genetic conservation approach. The plastid genome or nucleus, sometimes known as a universal DNA barcode, can be used to identify plant species. The *rbc*L, *mat*K, *trn*H-*psb*A intergenic spacer (IGS), and internal transcribed spacer (ITS) are some examples of often used regions (CBOL, 2009; Li et al., 2012; Balkanska et al., 2020). The ITS region has been evaluated as a DNA barcode system, yielding more diverse and informative characters (Rønsted et al., 2008; Li et al., 2012). The

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ITS region was tested and showed a quite clear cluster of *Homalomena* and *Philodendron*, separating both genus in each clade (Yeng et al., 2013).

DNA sequence data will provide an evolutionary measurement to diversity estimates by accounting for genetic distance between species. As a result, phylogenetic diversity can be assessed inside and across ecological communities at various geographic scales, used to aid in the documentation of new species, and used to select priority sites for conservation (Kress et al., 2015). They give a paradigm for conservation genomics and highlight key challenges from large-scale data sets (Govindaraj et al., 2015).

MATERIALS AND METHODS

Plant material preparation

The specimen used in this study was a personal living collection of *H. pexa* (MRH94, Figure 1) from South Tapanuli (Medan) grown in the glasshouse of the Research Center for Plant Conservation, Botanic Gardens, and Forestry – National Research and Innovation Agency. A healthy and fresh leaf was taken and placed in a tea bag. To ensure that the specimen dried completely, the tea bag was placed in a plastic bag packed with silica gel and left at room temperature for three days.

Figure 1. *Homalomena pexa*

DNA extraction, amplification, and sequencing

Homalomena pexa whole genomic DNA was obtained from the dried leaf using the Plant Genomic DNA Kit following the standard protocol provided by the manufacturer (Tiangen Biotech Co., Ltd., Beijing). The universal ITS primer following Sun et al. (1994) was used to amplify the region. A 50 µL reaction mixture containing 10 ng of the DNA template, 1 µM forward and reverse primer, 25 µL of MyTaq™ HS Red Mix (Bioline, USA), and 17 µL nuclease-free water was used for the PCR reaction. The PCR conditions of 1 cycle (95 °C for 3 min), 35 cycles (95 °C for 30 sec, 58 °C for 45 sec, and 72 °C for 45 sec), and 1 cycle (72 °C for 5 min) were performed. The PCR products were separated on a 1% agarose gel and photographed using a GelDoc UV trans-illuminator (BioRad). The PCR product was sent to 1st Base for the sequencing process through PT Genetika Science Indonesia service.

Sequence editing and phylogenetic tree construction

The forward and reverse ITS sequence were constructed into a contig sequence using the ClustalW method and then submitted to NCBI BLAST to determine the homology level (https://blast.ncbi.nlm.nih.gov/) (Hung and Weng, 2016). A total of 20 *Homalomena* sequences that emerged from the BLAST results were downloaded for later use in constructing phylogenetic trees (Table 1). In addition, the *Furtadoa mixta* sequence was also downloaded and treated as an outgroup. The phylogenic tree was constructed using the Neighbor-Joining (NJ) method and the Kimura 2-parameter model with 1000 bootstrap replicates (Kimura, 1980; Felsentein, 1985; Saitou and Nei, 1987). All the analysis was executed using MEGA X (Kumar et al., 2018).

RESULTS AND DISCUSSION

Homalomena pexa DNA samples were effectively extracted and amplified for 1040 base pairs using ITS primers (Figure 2). The amplified sequences included partial 18S ribosomal RNA gene sequences, complete ITS1 sequences, whole 5.8S ribosomal RNA gene sequences, complete ITS2 sequences, and partial 26S ribosomal RNA sequences.

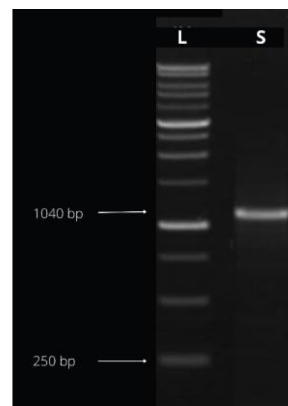

Figure 2. The amplified ITS sequence from *Homalomena pexa* (S) using 1 kb ladder (L) as a comparison.

Following BLAST result on the NCBI website, the ITS sequence for *H. pexa* had the highest sequence similarity with *H. atrox* (JQ955571.1) and

H. humilis (JQ413316.1), with 97.97% and 97.73%, respectively (Table 2). The high identity score showed that *H. pexa* from South Tapanuli is closely related to those two species.

The phylogenetic tree generated using ITS sequences revealed that all *Homalomena* species and outgroups are clustered together as a monophyletic clade (Figure 3). According to the phylogenetic tree, *H. pexa* is closely related to *H. atrox* and *H. humilis*, which is supported by a high bootstrap value (97%).

Generally, the ITS area amplified by the 17SE and 26SE primers can cover the region with sequences ranging from 573 to 1517 base pairs (Gehrig et al., 2001; Yeng et al., 2013; Hariri et al.,

Table 1. Sequence length variation of ITS region of *H. pexa* compared to NCBI database

No	Species	Accession No	Sequence Length (bp)
Observed specimen			
1	*Homalomena pexa*	This study	1040
NCBI Database Sequences			
Ingroup			
1	*Homalomena josefii*	JX076784.1	952
2	*Homalomena hanneae*	JX076779.1	946
3	*Homalomena sengkenyang*	JX076789.1	957
4	*Homalomena rostrata*	JX076786.1	922
5	*Homalomena vivens*	JX076796.1	924
6	*Homalomena clandestina*	JX076775.1	952
7	*Homalomena borneensis*	JQ413327.1	867
8	*Homalomena punctulata*	JX076785.1	900
9	*Homalomena insignis*	JX076783.1	915
10	*Homalomena havilandii*	JX076781.1	907
11	*Homalomena vagans*	JX076809.1	904
12	*Homalomena tonkinensis*	KM580741.1	965
13	*Homalomena rubescens*	KM580744.1	991
14	*Homalomena expedita*	JX076778.1	920
15	*Homalomena curvata*	JX076777.1	934
16	*Homalomena philippinensis*	DQ866881.1	876
17	*Homalomena humilis*	JQ413316.1	881
18	*Homalomena atrox*	JQ955571.1	835
19	*Homalomena asmae*	JQ413317.1	871
Outgroup			
1	*Furtadoa mixta*	KM580747.1	958

Table 2. Partial BLAST result of *H. pexa* based on top 10 percent of identity

BLAST result	Accession No	% ID	E value	Query Cover
Homalomena atrox	JQ955571.1	97.97%	0.0	79%
Homalomena humilis	JQ413316.1	97.73 %	0.0	83%
Homalomena philippinensis	DQ866811.1	96.37%	0.0	83%
Homalomena expedita	JX076778.1	95.84%	0.0	86%
Homalomena curvata	JX076777.1	95.64%	0.0	89%
Homalomena tonkinensis	KM580741.1	94.82%	0.0	91%
Homalomena rubescens	KM580744.1	94.76%	0.0	91%
Homalomena borneensis	JQ413327.1	94.52%	0.0	83%
Homalomena asmae	JQ413317.1	92.96%	0.0	83%
Homalomena insignis	JX076783.1	92.54%	0.0	87%

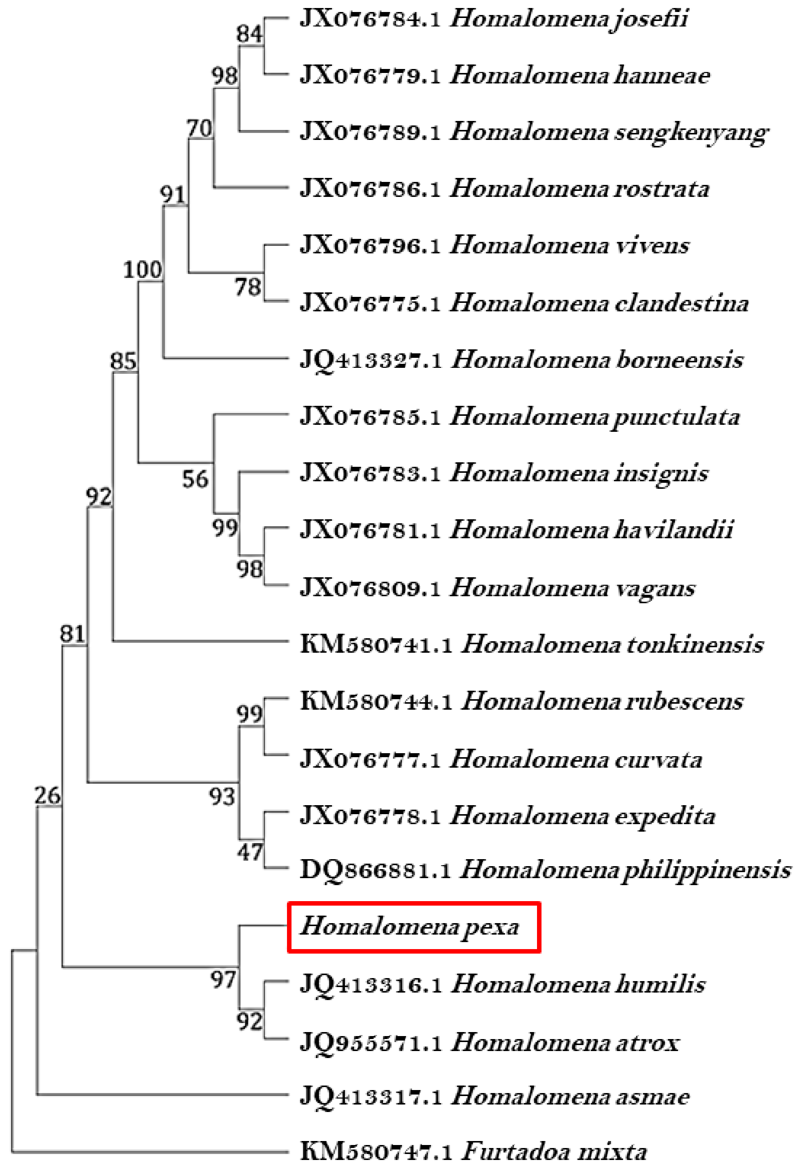

Figure 3. Dendrogram obtained from a Neighbor-Joining method with Kimura 2-parameter evolution model through 1000 bootstrap replications based on ITS sequence.

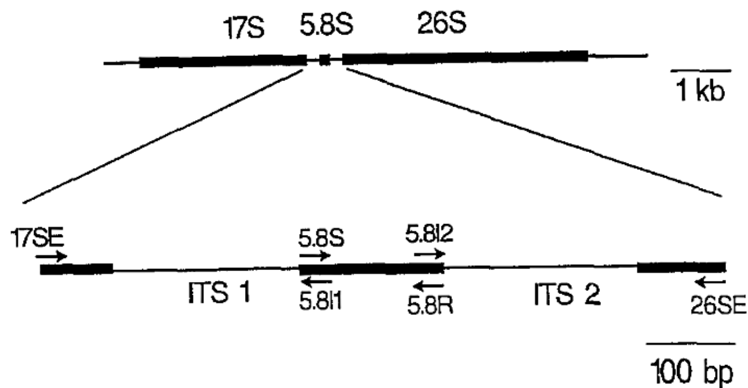

Figure 4. The full length of ITS region (Sun et al., 1994)

2021a; Hariri et al., 2021b; Irsyam et al., 2021) as shown in Figure 4. A relevant result provided by White *et al.* (1990) and Khisor and Devi (2009) revealed that a common length for ITS (ITS 1 dan ITS 2) ranged from 900 bp to 1500 bp covered ITS1, 5.8S rDNA dan ITS2. The length of the ITS1 and ITS2 regions varies slightly, with each length appearing to be genus-specific (Jobst et al., 1998). According to Yeng et al. (2013), the length of ITS1 ranged between 264 and 406 bp, the length of ITS2 varied between 326 and 395 bp, and the length of the 5.8S gene bp was consistent across all *Homalomena* taxa studied.

BLAST analyses revealed that ITS *H. pexa* sequences were 90% identical to *Homalomena*, 3% to *Furtadoa*, 6% to *Philodendron*, and 2% to *Adelonema*. These findings support the hypothesis that the sample sequence belongs to the genus *Homalomena*, which is supported further by the results of the phylogenetic tree construction. The clustering was built using the genetic distance, which grouped species with low genetic distances into their respective clades. Low genetic distance reveals that species within the same group are closely related (Clement and Donoghue, 2012).

Homalomena pexa is put in the same clade as *H. atrox* and *H. humilis* inside the *Homalomena* cluster, supported by a solid bootstrap value of 97%. Based on morphological characteristics, Wong et al. (2020) placed *H. pexa* in the same monophyletic group as *H. atrox* and *H. humilis*, namely the Chamaecladon clade. A species group is said to be monophyletic if all species found in the branches have a common ancestor (Podani, 2010). We used the same outgroup, *F. mixta*, to differentiate the relatonship among *Homalomena* species because *Furtadoa* and *Adelonema* are *Homalomena's* sister genera (Vasconcelos et al., 2018). Based on our findings, the ITS region has relatively significant interspecific variability and it is powerful enough to distinguish similar species among closely related species, including those in the *Homalomena* species complex (Jobst et al., 1998; Yeng et al., 2013; Hariri et al. 2021a; Irsyam et al. 2021). Given that Homalomena is one of the most speciose and taxonomically intractable aroid genera in the Asian tropics, the lack of a reliable taxonomy poses significant problems for field workers. A rigorous study aimed at resolving the taxonomy and phylogeny of the genus is desperately needed (Li & Boyce, 2010).

CONCLUSION

According to our findings, the ITS primer utilized in this investigation was successfully amplified and covered the entire ITS region. Using the core barcodes, for example, the *Homalomena* species complex, it could often unambiguously distinguish species belonging to the different major clades. We anticipate that comparable findings will be found in other plant groups. Moving forward, we encourage more data to be collected to expand the multilocus barcode to incorporate the extra markers required to accurately distinguish between closely related species.

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