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Analyses of Genetic Diversity and Relationships of Some *Polyscias* Samples Collected in Vietnam Using Morphological and Molecular Markers

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Abstract

The *Polyscias* genus belongs to the Araliaceae family and comprises over 116 species. This genus is grown in the Pacific and Southeastern Asian countries, and many of them possess great medicinal value. However, some *Polyscias* species in Vietnam are now endangered or threatened with extinction due to the high demand for medicinal use. The most popular *Polyscias* species, named "*Dinh lang*"(Ming aralia), have been largely cultivated for medicinal and ornamental purposes. Therefore, there is a need to develop both morphological and molecular markers to accurately classification. In this study, we collected 23 samples of the *Polyscias* J.R. Forst genus. & G. Forst. (Araliaceae) from 8 different regions (northern, central highland, and south-central coasts) in this country. All samples were characterized using morphological characteristics and ITS markers. As a result, 20 different phenotypes, 3 subspecies and 4 species were identified. Cluster analyses using similarity coefficients ranging from 98.38% to 100% showed that the 23 samples of *Polyscias* formed 4 major clusters with 12 genotypes. In general, morphological differences (mainly on leaves) and genetic characteristics using ITS markers were consistent with each other, and every genotype included one or more phenotypes. The study also identified the short keys to species and varieties for some *Polyscias* species growing in this country.

Keywords: Genetic diversity, *Polyscias*, species, *Dinh lang*, medicinal plant

1. Introduction

The *Polyscias* genus belongs to the Araliaceae family and comprises over 116 species. These species are commonly grown in the Pacific region and Southeastern Asia. Some species have been reported to have potential medicinal value. Specifically, approximately 100 chemical constituents have been isolated and identified. The major bioactive compounds were saponins with promising pharmacological activities such as anti-asthmatic, immuno-stimulant, antibacterial, antifungal, cytotoxic, and wound healing activities (Ashmawy et al., 2020). Vietnam is a tropical and subtropical country and is reported to be rich in plant diversity. The most popular *Polyscias* species, called " *Dinh lang*" in Vietnamese, has been popularly grown for a long time with both decorative and medicinal purposes in many localities across this country and is considered to possess great medicinal properties. However, many *Polyscias* species in Vietnam are now endangered or threatened with extinction due to the high demand for medicinal use.

The internal transcribed spacer (ITS) region of nuclear ribosomal DNA, including ITS1 and ITS2, has been widely applied in plant systematic to reconstruct phylogenetic relationships at different taxonomic levels (Baldwin, 1992; Trung et al., 2013). The ITS region is claimed to be useful for low-level phylogenetic analysis, such as infra-generic level, due to its relatively fast rate of evolution. On the other ways, ITS is a widely used molecular marker for inferring the phylogeny and genetic relatedness of angiosperms, and it is a valuable source of characters for phylogenetic studies in many plant species. Similarly, morphological traits have been studied for a long time, together with molecular markers, as an efficient method of species classification (Han et al., 2021). Therefore, the exploitation of morphological traits and molecular markers would lead to successful species classification.

In the last decade, numerous studies have been conducted on this species, most of them reported on identifying the structures of the detected compounds and examining the biological effects of certain species within the *Polyscias* genus (Chau et al., 2007; Long, 1977; Huong and Bich, 2001). Also, there have been some studies focusing on the morphological characteristics of *Polyscias guilfoylei* cv. *Quinquefolia* and *Polyscias fruticosa* (L.) Harms (Tuyen et al., 2019; Thuy et al., 2023), genetic polymorphism studies using the RAPD technique on *Polyscias fruticosa* (Tram and Luong, 2014), and studies on the identification of sequences of the *rbc*L gene of *Polyscias fruticosa*, *Polyscias guilfoylei*, and *Polyscias balfouriana* (Mai et al., 2019; Mai et al., 2020). Those studies have significantly contributed to identifying certain species within the *Polyscias* genus in Vietnam. A recent study has documented the morphological diversity of *Polyscias fruticosa* based on the morphological traits and sequences using *rbc*L marker (Mai et al., 2021). Unfortunately, there are few studies on genetic diversity and relationships on this species. Therefore, the objective of the current study was to assess the genetic diversity and the relationship of some *Polyscias* samples collected from different regions in this country using morphological and molecular markers.

2. Materials and Methods

2.1. Materials

In this study, a total of twenty-three *Polyscias* sample species were collected from different regions, including cultivated gardens, farmers' areas, supplying units, and research centres in this country as presented in Table 1.

Table 1. The information of the collected *Polyscias* samples used in this study

Nº	Collecting areas	Longitude (N) Latitude	The number of	Collected Sample ID	Sample ID for DNA	DNA ID corresponding
1	Nghia Lac commune, Nghia Hung district, Nam Dinh province	20°06'51.3"N 106°10'42.2"E	3	I1.1; I1.2; I1.3	LI1.1; LI1.2; LI1.3	DL1; DL2; DL3
2	Trau Quy town, Gia Lam district, Ha Noi city	20°56'40.5"N 105°47'69.0"E	4	I1.4 ; I1.5; I1.6; I1.7	LI1.4 ; LI1.5; LI1.6; LI1.7	DL4; DL5; DL6; DL7
3	Hai An commune, Hai Hau district, Nam Dinh province	20°08'36.8"N 106°11'33.1"E	7	II1.1; II1.2; III1.1; III1.2; III1.3; IV1.2; VI1.1 VI1.1; VI1.2	LII1.1; LII1.2; LIII1.1; LIII1.2; LIII1.3; LIV1.2; VI1.1 VI1.1; VI1.2	DL8; DL9; DL10; DL11; DL12; DL14; DL15; DL23
4	Hai Hoa commune, Hai Hau district, Nam Dinh province	20°04'21.7"N 106°14'48.3"E	1	IV1.1	LIV1.1	DL13
5	Tu Hiep commune, Thanh Tri district, Ha Noi city	20°99'48.2"N 105°8'33.6"E	3	VI1.3; IV1.3; VI1.2	LVI1.3; LIV1.3; LVI1.2	DL16; DL22; DL23
6	Chu Se town, Chu Se district, Gia Lai province	13°69'48.9"N 108°7'69.4"E	2	II2.1; II2.2	LII2.1; LII2.2	DL17; DL18
7	Hoa Hiep Nam town, Dong Hoa district, Phu Yen province	12°97′06.1”N 109°38′65.1”E	1	II3	LII3	DL19
8	Thach Dong commune, Thanh Thuy district, Phu Tho province	21°20'49.1"N 105°31'60.4"E	2	III4.1; III4.2	LIII4.1; LIII4.2	DL20; DL21

Note: Collected samples were whole plants with leaves, stems, roots and flowers. Samples for DNA were fresh leaves or leaves dried in silica gel.

2.2. Methods

All *Polyscias* samples were collected following the previously described method of On et al (2012) and Thuy et al (2022). Briefly, evaluation and classification criteria were based on the morphological characteristics of the 4th-5th leaves from the top-down view and/or the stem of the 2-year-old tree at a minimum. Leaf samples were subjected to DNA analysis using ITS-rDNA markers following the CTAB method by Doyle and Doyle (1987) and with some modifications (Ha et al., 2020). The primer sequences used were ITS-1: 5'-TCCGTAGGTGAACCTTGCGG-3' and ITS-8: 5'-GCACTACGATGAAGAACGCT3'. The scientific names of the botanical samples were identified by comparing their botanical characteristics with classification keys found in prominent botanical publications according to the methods of Xiang and Lowry (2007) and Robert (1992), and by referencing preserved type specimens housed in the specimen rooms of Kew Royal Botanic Garden, England (K) (2024) and the Chinese Virtual Herbarium (2024). The specimen samples were stored in the specimen room of the Department of Botany - Hanoi

University of Pharmacy (HNIP). The morphological characteristics were analyzed using PC-ORD, while the DNA sequences were analyzed using Geneious Prime® 2022.2.2.

3. Results and Discussion

In this study, 23 *Polyscias* samples were collected from different areas and provinces (8 places), including Hanoi, Nam Dinh, Gia Lai, Phu Yen, and Phu Tho provinces. In which, Gia Lai and Phu Yen are on the highland and south-central coast of Vietnam, while Hanoi, Nam Dinh and Phu Tho provinces are located in the northern areas of this country. Among the collected samples, 8 species with the name "Dinh lang" were narrated in the series of books on illustrated flora of Vietnam.

The 4th-5th leaves of 23 samples were selected to describe leaf characteristics and extract DNA using the ITS marker. Stems of young and mature trees were also observed and/or collected for morphological analysis data. A matrix of morphological characteristics was established for the 23 samples, including 33 variables (9 for stems, 11 for compound leaves, and 13 for leaflets). The classification results based on morphological characteristics analyzed with PC-ORD are presented in Figure 1. The classification tree showed that the 23 *Polyscias* samples, consisting of 21 phenotypes, were divided into 4 main groups based on less than 52.5% morphological similarities, as shown in Table 2.

Table 2. The list of groups based on the morphological similarities

Group	Scientific name	The similarity to the nearest neighbor group	The morphology similarities of samples in the group	
			The similarity coefficient	The similar samples
1A1	*Polyscias fruticosa* (L.) Harms	43.8%	67.9%	I1.5
			81.3%	I1.1; I1.7
			95.8%	I1.2; I1.3
1A2		14.3%	85.4%	I1.4; I1.6
2A	*Polyscias filicifolia* (C.Moore ex E.Fourn.) L.H.Bailey	52.5%	97.9%	II1.1; II2.1
			100.0%	II1.2; II2.2; II3
3A	*Polyscias guifoylei* (W.Bull) L.H.Bailey	35.7%	93.8%	III4.1
			96.9%	III1.1
			97.7%	III1.2; III4.2
4A	*Polyscias scutellaria* (Burm.f.) Fosberg	25.0%	75.0%	III1.3
			87.5%	IV1.1
			91.1%	VI1.1
			94.4%	VI1.2; VI1.3
			99.0%	IV1.2; IV1.3

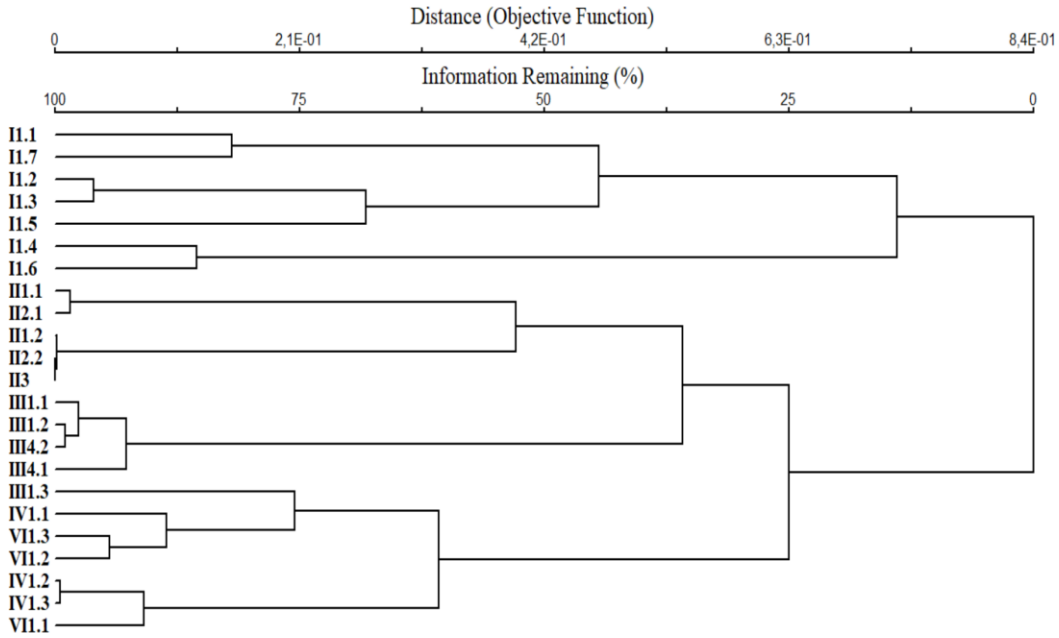

Figure 1. The classification tree based on the morphological characteristics

The results of DNA extraction from 23 *Polyscias* samples, which were tested by electrophoresis on a 1% agarose gel (Figure 2), indicated that the samples had good DNA quality without any impurities. Afterwards, the product was amplified using the primer pair ITS1/ITS4 and then electrophoresed on a 1.5% agarose gel, resulting in a monomorphic spectrum with a size of approximately 800 bp (Figure 3).

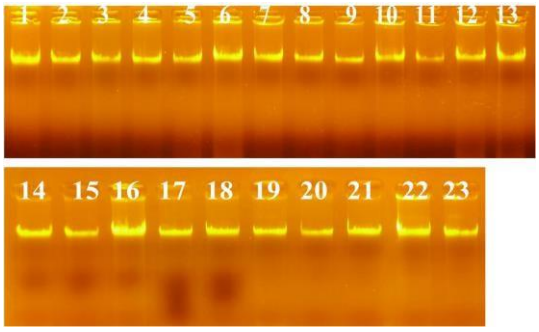

Figure 2. Total DNA electrophoresis image of 23 *Polyscias* samples

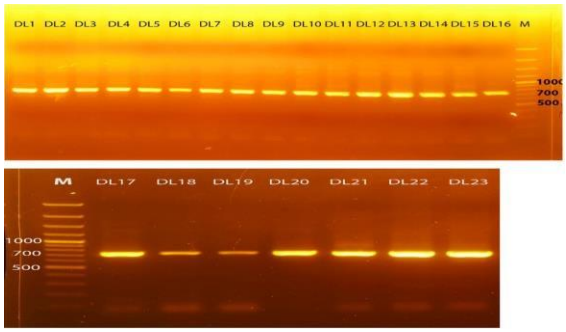

Figure 3. Electrophoresis of PCR products with primer pairs ITS1/ITS4 in 23 *Polyscias* samples with the standard marker: 1kb

The results of the ITS-rDNA sequences for the 23 *Polyscias* samples studied are presented in Table 3. These sequences belong to the general length and length of the ITS1-5.8SrRNA-ITS2 region. In which, the total number of nucleotide sequences was 731.0 nucleotides, including three samples (DL7, DL10 and DL18), and 4 samples were 733.0 (DL5, DL6, DL17 and DL19), and the nucleotides sequences of 16 samples were 732 (Dl1, Dl2, DL3, DL4, DL8, DL9, DL11, DL12, DL13, DL14, DL15, DL16, DL20, DL21, DL22 and DL23, respectively (Table 3).

Table 3. The length of DNA sequences of studied samples

Nº	DNA ID	Sample ID	Total number of nucleotides sequence	Nº	DNA ID	Sample ID	Total number of nucleotide sequence
1	DL1	LI1.1	732.0	13	DL13	LIV1.1	732.0
2	DL2	LI1.2	732.0	14	DL14	LIV1.2	732.0
3	DL3	LI1.3	732.0	15	DL15	LVI1.1	732.0
4	DL4	LI1.4	732.0	16	DL16	LVI1.3	732.0
5	DL5	LI1.5	733.0	17	DL17	LII2.1	733.0
6	DL6	LI1.6	733.0	18	DL18	LII2.2	731.0
7	DL7	LI1.7	731.0	19	DL19	LII3	733.0
8	DL8	LII1.1	732.0	20	DL20	LIII4.1	732.0
9	DL9	LII1.2	732.0	21	DL21	LIII4.2	732.0
10	DL10	LIII1.1	731.0	22	DL22	LIV1.3	732.0
11	DL11	LIII1.2	732.0	23	DL23	LVI1.2	732.0
12	DL12	LIII1.3	732.0				

Using Geneious software v.2022.02.02, all sequences were trimmed at the start of ITS1 and the end of ITS2 to minimise any noise that could affect the analysis. Consequently, a total of 23 ITS-rDNA sequences from the collected samples were compared to the similarity coefficients presented in Table 4.

Table 4. The similarity coefficients of 23 sample pairs

	LI1.1	LI1.2	LI1.3	LI1.4	LI1.5	LI1.6	LI1.7	LII1.1	LII1.2	LII2.1	LII2.2	LII3	LIII1.1	LIII1.2	LIII1.3	LIII4.1	LIII4.2	LIV1.1	LIV1.2	LIV1.3	LVI1.1	LVI1.2	LVI1.3
LI1.1		100%	100%	99.43%	99.92%	99.43%	100%	99.84%	99.68%	99.84%	99.76%	99.76%	99.51%	99.35%	98.70%	99.35%	99.51%	98.54%	98.54%	98.54%	98.54%	98.54%	99.11%
LI1.2	100%		100%	99.43%	99.92%	99.43%	100%	99.84%	99.68%	99.84%	99.76%	99.76%	99.51%	99.35%	98.70%	99.35%	99.51%	98.54%	98.54%	98.54%	98.54%	98.54%	99.11%
LI1.3	100%	100%		99.43%	99.92%	99.43%	100%	99.84%	99.68%	99.84%	99.76%	99.76%	99.51%	99.35%	98.70%	99.35%	99.51%	98.54%	98.54%	98.54%	98.54%	98.54%	99.11%
LI1.4	99.43%	99.43%	99.43%		99.35%	99.76%	99.43%	99.59%	99.59%	99.59%	99.68%	99.68%	99.76%	99.59%	98.62%	99.59%	99.76%	98.46%	98.46%	98.46%	98.46%	98.46%	99.19%
LI1.5	99.92%	99.92%	99.92%	99.35%		99.35%	99.92%	99.76%	99.68%	99.76%	99.68%	99.68%	99.43%	99.27%	98.62%	99.27%	99.43%	98.46%	98.46%	98.46%	98.46%	98.46%	99.03%
LI1.6	99.43%	99.43%	99.43%	99.76%	99.35%		99.43%	99.59%	99.59%	99.59%	99.68%	99.68%	99.76%	99.59%	98.62%	99.59%	99.76%	98.46%	98.46%	98.46%	98.46%	98.46%	99.19%
LI1.7	100%	100%	100%	99.43%	99.92%	99.43%		99.84%	99.68%	99.84%	99.76%	99.76%	99.51%	99.35%	98.70%	99.35%	99.51%	98.54%	98.54%	98.54%	98.54%	98.54%	99.11%
LII1.1	99.84%	99.84%	99.84%	99.59%	99.76%	99.59%	99.84%		99.84%	100%	99.92%	99.92%	99.68%	99.51%	98.87%	99.51%	99.68%	98.70%	98.70%	98.70%	98.70%	98.70%	99.27%
LII1.2	99.68%	99.68%	99.68%	99.59%	99.68%	99.59%	99.68%	99.84%		99.84%	99.84%	99.84%	99.68%	99.51%	98.70%	99.51%	99.68%	98.54%	98.54%	98.54%	98.54%	98.54%	99.19%
LII2.1	99.84%	99.84%	99.84%	99.59%	99.76%	99.59%	99.84%	100%	99.84%		99.92%	99.92%	99.68%	99.51%	98.87%	99.51%	99.68%	98.70%	98.70%	98.70%	98.70%	98.70%	99.27%
LII2.2	99.76%	99.76%	99.76%	99.68%	99.68%	99.68%	99.76%	99.92%	99.84%	99.92%		99.92%	99.76%	99.59%	98.78%	99.59%	99.76%	98.62%	98.62%	98.62%	98.62%	98.62%	99.27%
LII3	99.76%	99.76%	99.76%	99.68%	99.68%	99.68%	99.76%	99.92%	99.84%	99.92%	99.92%		99.76%	99.59%	98.78%	99.59%	99.76%	98.62%	98.62%	98.62%	98.62%	98.62%	99.27%
LIII1.1	99.51%	99.51%	99.51%	99.76%	99.43%	99.76%	99.51%	99.68%	99.68%	99.68%	99.76%	99.76%		99.68%	98.70%	99.68%	99.84%	98.54%	98.54%	98.54%	98.54%	98.54%	99.27%
LIII1.2	99.35%	99.35%	99.35%	99.59%	99.27%	99.59%	99.35%	99.51%	99.51%	99.51%	99.59%	99.59%	99.68%		98.87%	99.68%	99.68%	98.70%	98.70%	98.70%	98.70%	98.70%	99.27%
LIII1.3	98.70%	98.70%	98.70%	98.62%	98.62%	98.62%	98.70%	98.87%	98.70%	98.87%	98.78%	98.78%	98.70%	98.87%		98.87%	98.54%	99.51%	99.51%	99.51%	99.51%	99.51%	99.27%
LIII4.1	99.35%	99.35%	99.35%	99.59%	99.27%	99.59%	99.35%	99.51%	99.51%	99.51%	99.59%	99.59%	99.68%	99.68%	98.87%		99.68%	98.70%	98.70%	98.70%	98.70%	98.70%	99.27%
LIII4.2	99.51%	99.51%	99.51%	99.76%	99.43%	99.76%	99.51%	99.68%	99.68%	99.68%	99.76%	99.76%	99.84%	99.68%	98.54%	99.68%		98.38%	98.38%	98.38%	98.38%	98.38%	99.27%
LIV1.1	98.54%	98.54%	98.54%	98.46%	98.46%	98.46%	98.54%	98.70%	98.54%	98.70%	98.62%	98.62%	98.54%	98.70%	99.51%	98.70%	98.38%		100%	100%	100%	100%	98.95%
LIV1.2	98.54%	98.54%	98.54%	98.46%	98.46%	98.46%	98.54%	98.70%	98.54%	98.70%	98.62%	98.62%	98.54%	98.70%	99.51%	98.70%	98.38%	100%		100%	100%	100%	98.95%
LIV1.3	98.54%	98.54%	98.54%	98.46%	98.46%	98.46%	98.54%	98.70%	98.54%	98.70%	98.62%	98.62%	98.54%	98.70%	99.51%	98.70%	98.38%	100%	100%		100%	100%	98.95%
LVI1.1	98.54%	98.54%	98.54%	98.46%	98.46%	98.46%	98.54%	98.70%	98.54%	98.70%	98.62%	98.62%	98.54%	98.70%	99.51%	98.70%	98.38%	100%	100%	100%		100%	98.95%
LVI1.2	98.54%	98.54%	98.54%	98.46%	98.46%	98.46%	98.54%	98.70%	98.54%	98.70%	98.62%	98.62%	98.54%	98.70%	99.51%	98.70%	98.38%	100%	100%	100%	100%		98.95%
LVI1.3	99.11%	99.11%	99.11%	99.19%	99.03%	99.19%	99.11%	99.27%	99.19%	99.27%	99.27%	99.27%	99.27%	99.27%	99.27%	99.27%	99.27%	98.95%	98.95%	98.95%	98.95%	98.95%	

Table 4 shows that the similarity coefficients of 23 sample pairs are quite high, with the lowest value being 98.38% and the highest value 100%, respectively. The nucleotide differences in some single positions of the analyzed samples were expressed as the DNA sequence diversity, as shown in Table 5. Figure 4 displays the phylogenetic tree established using Geneious software and the Neighbor-joining method based on the nucleotide sequence of the ITS1-rRNA-ITS2 region. According to the taxonomic tree, which was based on the sequence of the ITS1-rRNA-

ITS2 region, the 23 samples of *Polyscias*, consisting of 12 genotypes (Table 5), were divided into 4 main groups as indicated in Table 6.

Table 5. Analyzing DNA diversity of *Polyscias* samples

No.	Collected sample ID	The position of difference nucleotide in 700 nucleotide sequences														Seq. ID	Other information (Sciencetific name base on the mophorlogy characteristic and literature)
		106	*114*	*162*	*199*	*207*	*404*	*450*	*463*	*475*	*481*	*487*	*493*	*630*	*676*		
1	LI1.1	C	G	T	A	T	G	G	G	T	A	G	G	C	G	*pfr*	These all were identified as *Polyscias fruticosa* (I), a very popular "Dinh lang' specy in Vietnam.
2	LI1.2	C	G	T	A	T	G	G	G	T	A	G	G	C	G		
3	LI1.3	C	G	T	A	T	G	G	G	T	A	G	G	C	G		
4	LI1.7	C	G	T	A	T	G	G	G	T	A	G	G	C	G		
5	LI1.5	C	G	T	A	T	G	G	G	T	A	G	G	C	R	*pfr1*	Identified as *Polyscias fruticosa* (I).
6	LII1.1	C	G	T	A	T	G	G	G	G	A	G	G	C	G	*pfi*	Identified as *Polyscias filicifolia* (II).
7	LII2.1	C	G	T	A	T	G	G	G	G	A	G	G	C	G		
8	LII1.2	C	S	T	A	T	G	G	G	G	A	G	G	C	R	*pfi1*	
9	LII2.2	C	S	T	A	T	G	G	G	G	A	G	G	C	G	*pfi2*	
10	LII3	C	S	T	A	T	G	G	G	G	A	G	G	C	G		
11	LIII4.2	C	C	T	A	T	G	G	G	G	A	A	G	C	G	*pgg*	Identified as *Polyscias guifoylei* (III).
12	LIII1.1	C	C	T	R	T	G	G	G	G	A	R	G	C	G	*pgg1*	
13	LIII1.2	Y	C	Y	R	T	G	G	G	G	A	R	G	C	G	*pgg2*	
14	LIII4.1	Y	C	Y	R	T	G	G	G	G	A	R	G	C	G		
15	LI1.4	C	C	T	R	T	R	G	G	G	A	R	G	C	G	*pfg*	Identified as *Polyscias fruticosa* with the characteristic like a hybrid between (I) and (III).
16	LI1.6	C	C	T	R	T	R	G	G	G	A	R	G	C	G		
17	LIV1.1	T	G	C	G	C	G	G	A	G	G	G	C	T	G	*psc*	Identified as *Polyscias scutellaria*.
18	LIV1.2	T	G	C	G	C	G	G	A	G	G	G	C	T	G		
19	LIV1.3	T	G	C	G	C	G	G	A	G	G	G	C	T	G		
20	LVI1.1	T	G	C	G	C	G	G	A	G	G	G	C	T	G		
21	LVI1.2	T	G	C	G	C	G	G	A	G	G	G	C	T	G		
22	LIII1.3	T	G	C	G	C	G	C	G	G	G	G	G	T	G	*psc1*	Identified as *Polyscias scutellaria*.
23	LVI1.3	Y	S	Y	R	Y	G	S	G	G	R	R	G	Y	G	*psc2*	Identified as *Polyscias scutellaria*.

Table 6. The list of groups based on the nucleotide sequence of ITS1-rRNA-ITS2

Group	Scientific name	Genetic interval of group	Genetic interval of samples in group	Group of samples
1B	*Polyscias fruticosa* (L.) Harms	0.0069	0	LI1.1 LI1.2 LI1.3 LI1.5 LI1.7
2B	*Polyscias filicifolia* (C.Moore ex E.Fourn.) L.H.Bailey	0.0042	0 1×10^{-6} 2×10^{-6} 3×10^{-6} 4×10^{-6}	LII1.1 LII1.2 LII2.1 LII2.2 LII3
3B1	*Polyscias fruticosa* (L.) Harms	0.0032	0 13.10×10^{-4}	LI1.4 LI1.6
3B2	*Polyscias guifoylei* (W.Bull) L.H.Balley		8.45×10^{-4} 15.70×10^{-4} 17.56×10^{-4} 19.83×10^{-4}	LIII4.2 LIII1.1 LIII4.1 LIII1.2
4B1	*Polyscias scutellaria* (Burm.f.) Fosberg	0.0036	0 0 0 0 0.003 0.0056	LIV1.1 LIV1.2 LIV1.3 LVI1.1 LVI1.2 LIII1.3
4B2		0	0	LVI1.3

This study analyzed 7 samples of *Polyscias fruticosa* (L.) Harms were selected based on morphology and cultivated areas in the northern areas of Vietnam. Among these samples, 7 phenotypes were identified. However, 5 of these phenotypes exhibited similar nucleotide sequences. This similarity was also observed in other studied *Polyscias* species. Furthermore, genotypic segregation within the same group was typically not more pronounced than phenotypic segregation within the same group (Table 7).

This suggests that a genotype may manifest as different phenotypes depending on the specific growing or natural adapting conditions. The classification based on morphological characteristics (a) identified in plant taxonomy literature was slightly different from the classification based on molecular characteristics (b) and the classification based on keys to species and cultivars (c). There were three species, namely *Polyscias filicifolia* (C.Moore ex E.Fourn.) L.H.Bailey – *'Dinh lang la rang'*, in Vietnamese, *Polyscias fruticosa* (L.) Harms – *'Dinh lang la nho'*, and *Polyscias guifoylei* (W.Bull) L.H.Balley – *'Dinh lang la tro'*, that have a close genetic relationship, which was suitable to the keys to species in the *Polyscias* genus, as stated in the Flora of Malesiana (Philipson and Christchurch, 1979). Two taxa, I1.4 and I1.6, initially classified as *Polyscias fruticosa* (L.) Harms, based on the morphological traits, were later clustered into the group and named *Polyscias guilfoylei* (W.Bull) L.H.Balley based on the nucleotide sequence of the ITS region.

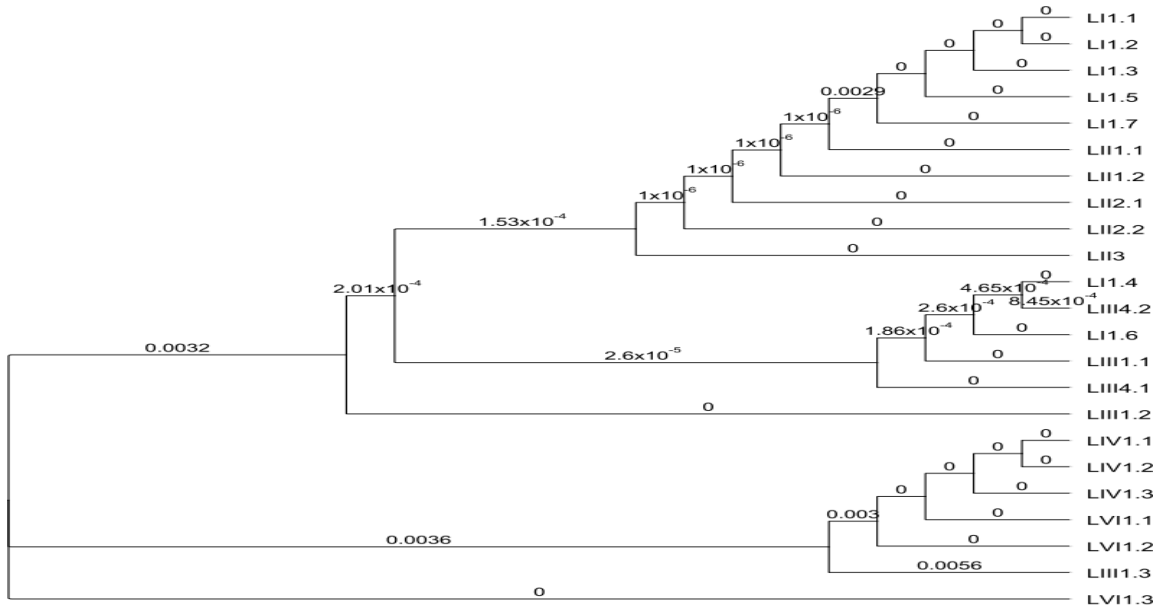

Figure 4. Phylogenetic tree based on the nucleotide sequence of the ITS1-rRNA-ITS2 region

Table 7. Phenotypes and genotypes of *Polyscias* species in Vietnam

No	Scientific name	NP*	NG	Typical morphology characteristics
1	*Polyscias fruticosa* (L.) Harms	7	3	- Compound leaves with 1-3 pinnate. Leaflets are often variegated. - Compound leaves with 1-2 pinnate: 2 samples have the genetic characteristic similar to *Polyscias guifoylei* (W.Bull) L.H.Balley the most.

2	*Polyscias filicifolia* (C.Moore ex E.Fourn.) L.H.Bailey	3	3	Compound leaves with 1 pinnate, margins of leaflets entire to coarsely crenate, blades elliptic to oblong (Xiang and Lowry, 2007).
3	*Polyscias guifoylei* (W.Bull) L.H.Balley	3	3	Compound leaves with 1 pinnate, margins of leaflets sharply serrulate (Xiang and Lowry, 2007); leaves commonly with 5 or 7 leaflets; margins of leaflets sharply serrulate (Robert, 1992).
4	*Polyscias scutellaria* (Burm.f.) Fosberg	7	3	Compound leaves often seem simple (unifoliolate), to 3-foliolate or 1-pinnate; leaflets up to 2 pairs on compound leaves, ovate, orbicular or cordate, obtuse, concave and saucer-like, dentate, green, sometimes with a white margin.

*Note: NP: Number of phenotypes; *: Appendixes (1S, 2S, 3S, 4S, 5S) illustrated the phenotypes of samples in detail; NG: the number of genotypes based on the nucleotide sequence of the ITS region.*

Three taxa (IV1.1, IV1.2, and IV1.3) were usually classified as *Polyscias balfouriana* (André) L.H.Bailey – *'Dinh lang la tron*,' while 2 others were classified as *Polyscias scutellaria* (Burm.f.) Fosberg – *'Dinh lang la dia*.' Additionally, 1 taxon (III1.3) was classified as *Polyscias guilfoylei* cv. *quinquefolia* (Bull) L.H. Bailey – *'Dinh lang rang*,' showing a high genetic coefficient. The results indicate a similarity between the phylogenetic tree and the key species in Flora of China and Flora of Malesiana. Therefore, all 7 taxa (IV1.1, IV1.2, IV1.3, VI1.2, VI1.2, VI1.3, and III1.3) were identified as *Polyscias scutellaria* (Burm.f.) Fosberg based on the nucleotide sequence of the ITS region. However, III1.3 was considered as *Polyscias guilfoylei* cv. *quinquefolia* (Bull) L.H. Bailey according to the cultivar keys in Ornamental Garden Plants of the Guianas (Robert, 1992).

Indeed, advanced genetic methods for classifying species, such as generating the phylogenetic tree, are based on the nucleotide sequence of the ITS region. However, the phylogenetic diversity and morphology diversity mentioned above did not completely align with the keys to species and cultivars. Furthermore, there was a 4.3% difference in the species ratio between (a) and (c) that was related to III1.3. Additionally, there was an 8.7% percentage difference between (a) and (b) that was related to I1.4 and I1.6. The difference ratio between (b) and (c) was 13.0%, which was related to I1.4, I1.6, and III1.3. Moreover, the classification of III1.3 according to both (a) and (b) yields the same result as the dendrogram of cluster analysis using RAPD markers in 2007 (Rout et al., 2007). Therefore, when the morphology-based classification has not been fully suitable for species identification because of the interaction between genetics and the environment in nature, the ITS phylogenetic characteristics may be a better choice for identifying the scientific name of *Polyscias* species.

In addition, it notes that I1.4 and I1.6 are possibly expected phenotypes of a new '*Dinh lang*' species in Vietnam. More information on botanical clues, such as flower and their morphology variation, as well as genetic changes according to the time, growing conditions and different regions, need to be further validated.

4. Conclusions

In Conclusion, the *Polyscias* species samples collected in different areas in Vietnam exhibit a wide range of morphological variations, which can be classified into four main groups

consisting of 20 phenotypes. The first group, *P. fruticosa* (L.) Harms *'Dinh lang la nho*,' comprises 7 phenotypes. The second group, *P. filicifolia* (C.Moore ex E.Fourn.) L.H.Bailey) *'Dinh lang la to*,' consists of 3 phenotypes. The third group, *P. guilfoylei* (Bull) L.H. Bailey) *'Dinh lang la tro*,' has 3 phenotypes. Finally, the fourth group, *P. scutellaria* (Burm.f.) Fosberg, comprises 7 phenotypes, three of which were usually identified as *P. balfouriana* (André) L.H.Bailey) - synonym of *P. scutellaria* (Burm.f.) Fosberg - and may be redefined as *P. scutellaria* (Burm.f.) Fosberg cv. balfouriana. Additionally, there were 3 phenotypes known as *'Dinh lang la dia*,' which could be redefined as *P. scutellaria* (Burm.f.) Fosberg cv. *scutellaria*, and one phenotype is known as *'Dinh lang rang*,' which may be redefined as *P. scutellaria* (Burm.f.) Fosberg cv. *quinquefolia*. These *Polyscias* species are currently valuable genetic resources that provide materials for selecting herbal medicinal seeds and producing ingredients from medicinal herbs under the name of "*Dinh lang*" in Vietnam. In terms of genetic diversity, the study documented the 12 different ITS-rADN sequences among the 23 collected samples of *Polyscias* genus. A phylogenetic tree was constructed based on these ITS-rADN sequences, and some sequences are being registered on GenBank on PubMed. Overall, the morphological diversity observed in the collected *Polyscias* samples aligns with their genetic characteristics as indicated by the ITS markers, although there have been slight differences from the existing keys to species. Therefore, it is necessary to establish new keys to distinguish *Polyscias* species planted in Vietnam for effective conservation, development and exploitation.

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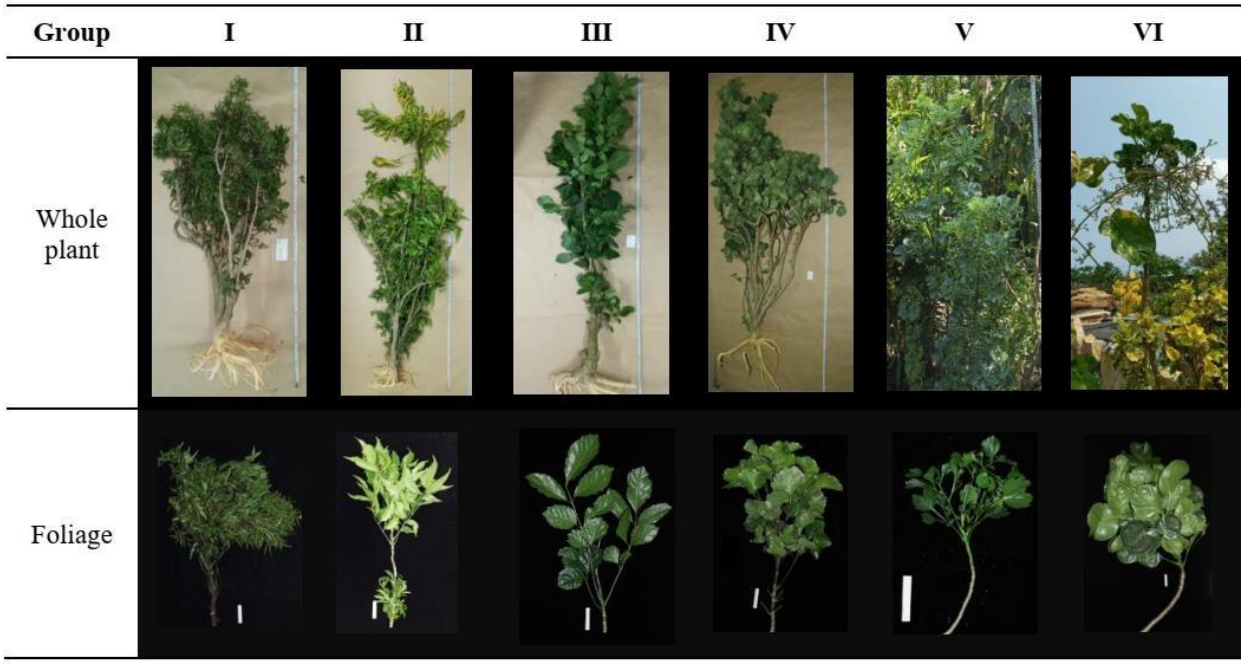
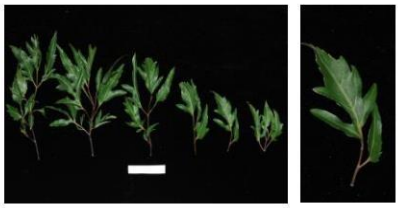
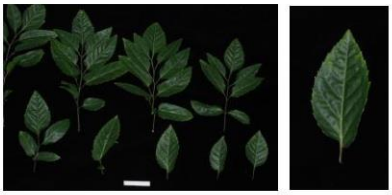
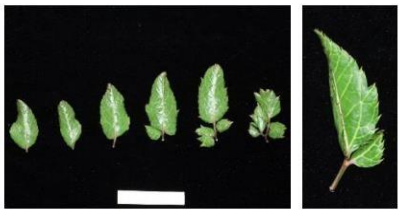
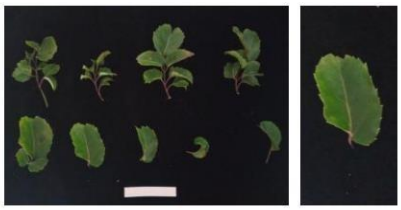
Appendix 1S. Images of typical whole plant and foliage in initial groups (*scale bar: 5cm*)



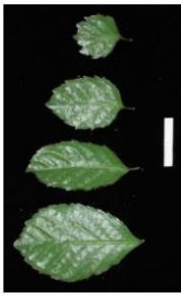
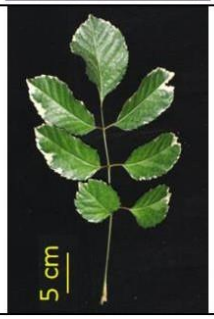
Appendix 2S. Images and morphology characteristics of leaves samples in Group 1

Sample Code	Compound leaves		Pinnate	*Scale bar: 5 cm*
	Type	**Image**	**Shape and colour**	**Image**
LI1.1	2-3-pinnately compound.		Narrow ellipse, divided into many lobes, with many lobes at the tip of the pinnate. Green.	
LI1.2	2-pinnately compound.		Wide ellipse, oval or inverted ovate, not lobed. Dark green.	
LI1.3	2-pinnately compound.		Wide ellipse, slightly lobed at the petiole or not lobed, with wrinkled leaf edges. Dark green.	
LI1.4	1-2-pinnately compound.		Narrow ellipse, thick, curved along the leaf, clear veins, and the leaf base is often crooked on one side. Green.	
LI1.5	1-2-pinnately compound.		Wide ellipse, nearly round and split lobe at the apex pinnate. Light green.	
LI1.6	2-3-pinnately compound.		Narrow ellipse, deeply lobed close to the leaf veins, creating small and long pieces. Light green.	
LI1.7	1-3-pinnately compound.		Narrow ellipse, divided into many lobe s, with many lobes at the tip of pinnate. Green.	

Appendix 3S. Images and morphology characteristics of leaves samples in Group 2

Sample Code	Compound leaves		*Scale bar: 5cm*			Pinnate
	Type	Image				Shape and colour
LII1.1, LII2.1	1- pinnately compound.					Oblong or narrow lanceolate, not lobed or very lightly lobed. Yellow-green.
LII1.2, LII2.2	1- pinnately compound.					Oblong or narrow lanceolate, divided into many feather-shaped lobes. Yellow-green.
LII3	1-pinnately compound					Narrow ellipse, not lobed. Yellow-green.

Appendix 4S. Images and morphology characteristics of leaves samples in Group 3

Sample Code	Compound leaves			Pinnate	*Scale bar: 5 cm*
	Type	**Image**		**Shape and colour**	**Image**
LIII1.1, LIII4.1	1- pinnately compound, leaflets (5)–7–9, often variegated.			Inverted ovoid or nearly round shape. Dark green.	
LIII1.2	1- pinnately compound, leaflets (5)–7–9, often variegated.			Nearly round shape. Dark green, with thick white or ivory white leaf edges.	
LIII4.2	1- pinnately compound, leaflets (5)–7–9, often variegated.			Inverted ovoid or nearly round shape. Dark green, with white or ivory white leaf edges.	

Appendix 5S. Images and morphology characteristics of leaves sample in Group 4

Sample Code	Compound leaves			Pinnate	*Scale bar: 5 cm*
	Type	**Image**		**Shape and colour**	**Image**
LIV1.1	Compound, leaflets 1-3.			Round or nearly round oval. Pale yellow-green color, with dark green leaf edges.	
LIV1.2	Compound, leaflets 1-3.			Round or nearly round oval. Green color, with ivory white edges.	
LIV1.3	Compound, leaflets 1-3.			Round or nearly round oval shape. Green with wide ivory-white edges, rarely whole pale ivory.	
LVI1.1	Compound, leaflets 1, 3.			Blades broadly elliptic to oblate or reniform, apex rounded. Dark green.	
LVI1.2	Compound, leaflets 1 (leaves unifoliolate), 3, or 5 (rarely 2 or 4).			Blades broadly elliptic to oblate or reniform, apex rounded. Dark green.	
LIII1.3	Compound, leaflets 3, 5.			The central leaflet is kidney-shaped, the base leaflet is often inverted ovoid shape and deeply split, long secondary cover, the edge of the leaflet is split into 2 - 4 section and winding	
LVI13	Compound, leaflets 1 (leaves unifoliolate), 3, or 5 (rarely 2 or 4).			Blades broadly elliptic to oblate or reniform, apex rounded. The uper side of the leaf is green, the lower side is purple.