



Original Article

# A Molecular Phylogeny of *Panax* L. Genus (Araliaceae) Based on ITS-rDNA and *matK* Support for Identification of *Panax* Species in Vietnam

Pham Thi Ngoc<sup>1</sup>, Pham Thanh Huyen<sup>1,\*</sup>, Nguyen Quynh Nga<sup>1</sup>, Phan Van Truong<sup>1</sup>,  
Nguyen Minh Khoi<sup>1</sup>, Dinh Doan Long<sup>2</sup>

<sup>1</sup>National Institute of Medicinal Materials, Ministry of Health  
3B Quang Trung Street, Hoan Kiem, Hanoi, Vietnam

<sup>2</sup>VNU School of Medicine and Pharmacy, Vietnam National University, Hanoi  
144 Xuan Thuy, Cau Giay, Hanoi, Vietnam

Received 21 January 2020

Revised 26 April 2020; Accepted 20 June 2020

**Abstract:** *Panax* L. is a small genus in the Araliaceae family. In Vietnam, this genus is distributed in the high mountains in the North and in some high mountains in the Central Highlands. In traditional medicine, roots and rhizomes of all *Panax* species are of high utility. Recently, the finding of new distributions of some *Panax* species in Vietnam has caused much controversy and confusion. This study investigates 27 fresh specimens of 6 taxa of *Panax* genus that have been collected from 6 provinces of Vietnam. Based on the combined ITS-rDNA and *matK* dataset, a well-resolved phylogeny of *Panax* species/varieties distributed in Vietnam was constructed. Thereby, the study suggests identifying Tam that la xe as *Panax stipuleanatus* H.T.Tsai et K.M.Feng and Sam puxailaileng as *Panax vietnamensis* var. *fuscidiscus* K.Komatsu, S.Zhu & S.Q.Cai, which contributes a new distribution point of this variety in Vietnam. The study also shows that ITS-rDNA and *matK* genes are highly potential in identifying and distinguishing the taxa of *Panax* genus.

**Keywords:** *Panax*, ginseng, molecular phylogeny, taxonomy, ITS-rDNA, *matK*.

## 1. Introduction

*Panax* is a small genus of Araliaceae family, comprise about 16-18 species worldwide,

distribution with a concentration in the Eastern Asia, Central to Eastern Himalayan region, China, South-East Asia and North America [1].

\* Corresponding author.

Email address: [huyenptnimm@gmail.com](mailto:huyenptnimm@gmail.com)

<https://doi.org/10.25073/2588-1132/vnumps.4198>

In Vietnam, there are three species and two varieties of *Panax* have been recorded, consist of: Tam that hoang - *P. stipuleanatus* H. T. Tsai et K. M. Feng; Sam ngoc linh - *P. vietnamensis* Ha et Grushv. with two varieties Sam lai chau - *P. vietnamensis* var. *fuscidiscus* K.Komatsu, S. Zhu & S.Q.Cai, Sam langbian - *P. vietnamensis* var. *langbianensis* N.V. Duy. V.T. Tran & L.N. Trieu, and Tam that - *P. notoginseng* (Burkill) F.H.Chen. (cultivated species). *Panax* species have narrow distribution, concentrated in some high mountain ranges in the Northern (Tay Con Linh, Hoang Lien Son, Pu Si Lung, Pu Sam Cap moutain), in the Central (Pu Xa Lai Leng and

Ngoc Linh mountain) and Central Highland (Langbian moutain) [2-5]. All of the *Panax* species are high value medicinal plants with various chemical composition [6]. Thus, authentication these species are very important for conservation and development these genetic resources.

Along with morphological method, the using of DNA barcode to classify *Panax* species are more and more popular over the world. Recently, a lot of study on phylogenetic relationship of *Panax* genus using the combination of DNA barcodes belong to chromosome and chloroplast genome were performed [7-12].

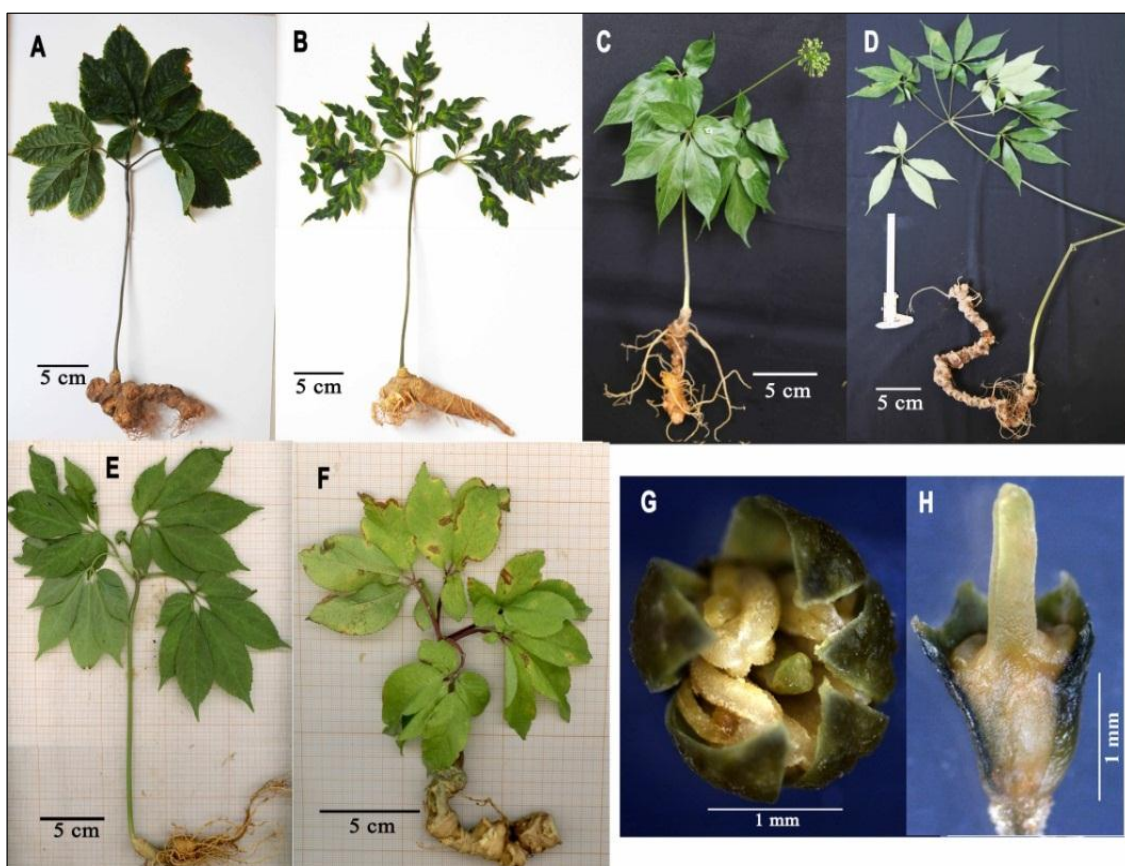


Figure 1. Representation of the samples in this study.

(A) Tam that hoang - *Panax stipuleanatus* Tsai & Feng; (B) Tam that la xe - *Panax* sp.1; (C) Sam ngoc linh - *Panax vietnamensis* Ha & Grushvitzky; (D) Sam lai chau - *Panax vietnamensis* var. *fuscidiscus* K.Komatsu, S.Zhu & S.Q.Cai; (E) Tam that bac - *Panax notoginseng* (Burkill) F.H.Chen; (F) Sam puxailaileng – *Panax* sp.2, with (G) flower and (H) flat disk flower base on longitudinal section of ovary. (Photo A-H taken by Pham Thi Ngoc; A-B: 7/2015; C: 7/2015; D: 7/2017; E: 5/2017; F: 9/2017 G-H: 7/2017).

In Vietnam, there were some studies base on molecular markers to study the phylogenetic relationship of *Panax* species aim to identify [5,13-16] or record new taxon [4,17]. The molecular markers used for analysis consist of DNA fragments in the chromosome and chloroplast genome as 18S rRNA, ITS, *psbA-trnH*, *matK*, *trnL*, *rbcL*, *rpoB*,... Recently, Manzanilla et al. (2018) have used a full plastome phylogenomic framework to resolve relationships in *Panax* and to identify molecular markers for species discrimination [12]. However, these studies had been carried out on one or few species/varieties instead of all the *Panax* species/varieties recorded in Vietnam. In particular, it has not paid attention to the samples of Tam that la xe which previously identified as *P. bipinnatifidus* Seem. by Wu Cheng Yi but laterly considered to be more similar to *P. stipuleanatus* by Pham Thi Ngoc et al. (2017) [18]. Tam that la xe possess most of the same morphological characteristics to *P. stipuleanatus* (bamboo-like rhizome, concave flower disk,...) and differ to this species only in pinnate leaflet. In addition, the samples of Sam puxailaileng (Pu Xai Lai Leng mountain, Ky Son dist., Nghe An prov.) were identified as *P. vietnamensis* based

on ITS-rDNA sequence [5] need further studies with more DNA markers.

In this study, we analyze the phylogenetic relationship based on the combined ITS-rDNA and *matK* sequences among all the *Panax* species/varieties in Vietnam for species/varieties discrimination.

## 2. Materials and methods

### 2.1. Materials

Total of 27 fresh samples were collected and dried in sealed bags with silica-gel, stored in the laboratory, where each sample was preserved at a constant -20 °C for DNA analysis. The samples from four species/varieties and two unidentified *Panax* taxa (“Tam that la xe” - *Panax* sp.1 and “Sam Puxalaileng” - *Panax* sp.2) were collected in 6 provinces in Northern and Central of Vietnam: Lao Cai, Ha Giang, Lai Chau, Nghe An, Quang Nam, Kon Tum (Table 1). All specimens were stored in the herbarium of medicinal materials, National Institute of Medicinal Materials (NIMM).

Table 1. Sampling information

Collection number	Vietnamese common name	Scientific name	Collected location	Collection date	Collector
P.01	Sam ngoc linh	<i>P. vietnamensis</i> Ha et Grushv.	Tak Ngo, Tra Linh, Nam Tra My, Quang Nam	06/07/2014	Nguyen Xuan Nam
P.02	Sam ngoc linh	<i>P. vietnamensis</i> Ha et Grushv.	Tak Ngo, Tra Linh, Nam Tra My, Quang Nam	23/07/2014	Nguyen Xuan Nam
P.03	Sam ngoc linh	<i>P. vietnamensis</i> Ha et Grushv.	Mang Ruong 2, Ngoc Lay, Tu Mo Rong, Kon Tum	27/07/2014	Nguyen Xuan Nam
P.04	Sam ngoc linh	<i>P. vietnamensis</i> Ha et Grushv.	Tra Linh, Nam Tra My, Quang Nam	28/11/2014	Nguyen Xuan Nam
P.05	Sam ngoc linh	<i>P. vietnamensis</i> Ha et Grushv.	Mang Lung, Tra Linh, Nam Tra My, Quang Nam	20/07/2015	Nguyen Xuan Nam
P.06	Sam ngoc linh	<i>P. vietnamensis</i> Ha et Grushv.	Mang Lung, Tra Linh, Nam Tra My, Quang Nam	20/07/2015	Nguyen Xuan Nam
P.07	Sam ngoc linh	<i>P. vietnamensis</i> Ha et Grushv.	Mang Ruong 2, Ngoc Lay, Tu Mo Rong, Kon Tum	23/07/2015	Nguyen Xuan Nam

P.08	Sam ngọc linh	<i>P. vietnamensis</i> Ha et Grushv.	Ko Xia II, Ngoc Lay, Tu Mo Rong, Kon Tum	24/07/2015	Nguyen Xuan Nam
P.09	Tam that la xe	<i>Panax</i> sp.1	Ban Khoang, Sa Pa, Lao Cai	13/07/2015	Pham Thi Ngoc
P.10	Tam that la xe	<i>Panax</i> sp.1	Ho Thau, Hoang Su Phi, Ha Giang	12/06/2016	Phan Van Truong
P.11	Tam that la xe	<i>Panax</i> sp.1	Ho Thau, Hoang Su Phi, Ha Giang	12/06/2016	Phan Van Truong
P.12	Tam that la xe	<i>Panax</i> sp.1	Ho Thau, Hoang Su Phi, Ha Giang	30/05/2017	Phan Van Truong
P.13	Tam that la xe	<i>Panax</i> sp.1	Ho Thau, Hoang Su Phi, Ha Giang	30/05/2017	Phan Van Truong
P.14	Tam that la xe	<i>Panax</i> sp.1	Ta Phin, Sa Pa, Lao Cai	08/08/2017	Pham Ngoc Khanh
P.15	Tam that hoang	<i>P. stipuleanatus</i> H.T.Tsai et K.M. Feng	Ban khoang, Sa Pa, Lao Cai	13/07/2015	Pham Thi Ngoc
P.16	Tam that hoang	<i>P. stipuleanatus</i> H.T.Tsai et K.M. Feng	Ho Thau, Hoang Su Phi, Ha Giang	12/06/2016	Phan Van Truong
P.17	Tam that hoang	<i>P. stipuleanatus</i> H.T.Tsai et K.M. Feng	Ho Thau, Hoang Su Phi, Ha Giang	12/06/2016	Phan Van Truong
P.18	Tam that hoang	<i>P. stipuleanatus</i> H.T.Tsai et K.M. Feng	Ho Thau, Hoang Su Phi, Ha Giang	30/05/2017	Phan Van Truong
P.19	Tam that hoang	<i>P. stipuleanatus</i> H.T.Tsai et K.M. Feng	Ta Phin, Sa Pa, Lao Cai	08/08/2017	Pham Ngoc Khanh
P.20	Sam lai chau	<i>P. vietnamensis</i> Ha & Grushv. var. <i>fuscidiscus</i> K.Komatsu, S. Zhu & S.Q.Cai	Muong Te, Lai Chau	24/04/2017	Phan Van Truong
P.21	Sam lai chau	<i>P. vietnamensis</i> Ha & Grushv. var. <i>fuscidiscus</i> K.Komatsu, S. Zhu & S.Q.Cai	Sin Ho, Lai Chau	27/07/2017	Phan Van Truong
P.22	Sam lai chau	<i>P. vietnamensis</i> Ha & Grushv. var. <i>fuscidiscus</i> K.Komatsu, S. Zhu & S.Q.Cai	Sin Ho, Lai Chau	27/07/2017	Phan Van Truong
P.23	Sam lai chau	<i>P. vietnamensis</i> Ha & Grushv. var. <i>fuscidiscus</i> K.Komatsu, S. Zhu & S.Q.Cai	Sin Ho, Lai Chau	27/07/2017	Phan Van Truong
P.24	Sam lai chau	<i>P. vietnamensis</i> Ha & Grushv. var. <i>fuscidiscus</i> K.Komatsu, S. Zhu & S.Q.Cai	Muong Te, Lai Chau	19/09/2017	Phan Van Truong
P.25	Sam puxailaileng	<i>Panax</i> sp.2	Muong Long, Ky Son, Nghe An	14/07/2017	Phung Van Hao
P.26	Sam puxailaileng	<i>Panax</i> sp.2	Muong Long, Ky Son, Nghe An	29/09/2017	Phan Van Truong
P.27	Tam that bac	<i>Panax notoginseng</i> (Burkill) F.H.Chen	Hoang Su Phi, Ha Giang	30/05/2017	Phan Van Truong

## 2.2. Methods

### 2.2.1. DNA extraction, PCR amplification, DNA purification and sequencing

Total DNA was extracted from dried leaves of those samples, using a Qiagen Dneasy plant extraction kit (Qiagen, Germany) with the provided protocol. Complete ITS-rDNA region gene and partial *matK* gene amplifications via the polymerase chain reaction (PCR) were performed using 5 µl (10-50 ng) total DNA as a template in 25 µl reaction mixture, containing 0.5 µM of each primer, 2.5 µl dNTP mix, 2.5 µl DreamTaq Buffer 10X, 0.625 U DreamTaq DNA polymerase (ThermoFisher Scientific, USA) and deionized water. For application of the ITS region amplification, the primers used were C26A (5'-GTT TCT TTT CCT CCG CT-3') and Nnc18S10 (5'-AGG AGA AGT CGT AAC AAG-3') [8]; thermal cycling conditions were as follows: a sample denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94°C for 50 sec, primer annealing at 54°C for 55 sec, primer extension at 72°C for 2 min, and final extension step at 72°C for 10 min. For partial *matK* gene amplification, the primers used were *matK* AF (5'-CTA TAT CCA CTT ATC TTT CAG GAG T-3') and *matK* 8R (5'-AAA GTT CTA GCA CAA GAA AGT CGA-3') (Ooi et al. 1995); thermal cycling conditions were as follows: a sample denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 2 min and primer annealing at 62°C for 1 min and primer extension at 72°C for 2 min, and final extension step at 72°C for 10 min. PCR products were then purified using QIAquick Gel Extraction Kit (Qiagen, Germany). Sanger sequencing was performed on an ABI 3730 Genetic Analyzer system using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA).

### 2.2.2. Phylogenetic analysis

The newly generated sequences were assembled by Geneious version 6.0.6 (<http://www.geneious.com>, Biomatters, 2013). Nucleotide sequences were aligned using MUSCLE 3.8.31 (Edgar, 2004). Sequences from

the sampled taxa of the genus *Panax* collected in this study along with those from Genbank and of the outgroup taxa were examined for sequence comparisons and phylogenetic trees reconstruction based on two dendrogram building methods: Maximum Likelihood (ML) in RaxML 8.2.8 (Stamakis, 2014) and Bayesian Inference (BI) in MrBayes v.3.2.6 (Ronquist và Huelsenbeck, 2003) on the CIPRES Science Gateway (Miller et al., 2010) under the GTR + G model with 1000 replications.

*Supporting information:* Genbank accession numbers of 45 DNA sequences of ITS-rRNA and 45 sequences of *matK* belonging to 13 taxa that distributed in 6 countries and territories consist of American, China, Japan, Nepal, Taiwan and Vietnam.

## 3. Results and discussion

### 3.1. ITS-rDNA and *matK* sequences analysis

Obtained partial of ITS-rDNA and *matK* gene sequences of the samples were found to be 702 - 715 and 1061 - 1104 base pairs in length respectively. Sequences of the samples in the same taxon were similar suggesting the intra-species stability of *Panax* species on ITS-rDNA and *matK* gene sequences. Comparison among eight taxa of *Panax* occurred in Vietnam found out the differences in 40 positions on ITS-rDNA and 27 positions on *matK* gene (Table 2 & 3). This results show that the ITS-rDNA and *matK* gene are high potential in distinguishing those taxa of *Panax* genus in Vietnam.

Comparing the ITS-rDNA and *matK* gene sequences between Tam that hoang (*Panax stipuleanatus*) and Tam that la xe (*Panax* sp.1) samples, we found that all the samples divided into two groups related to the two collected sites: Lao Cai and Ha Giang province. Samples in the same group were 100% identical in ITS-rDNA and *matK* gene sequences while samples in different groups are distinguished by nucleotide position 472 on ITS-rDNA gene and nucleotide position 462 on *matK* gene. Group 1 including all the samples collected in Lao Cai (Sa Pa)

which belong to both Tam that hoang - *Panax stipuleanatus* and unidentified Tam that la xe - *Panax* sp.1 (P15, P19 and P09, P14 respectively). Group 2 including the other samples collected in Ha Giang which also belong to both Tam that hoang - *Panax stipuleanatus*

and unidentified Tam that la xe - *Panax* sp.1 (P16, P17, P18 and P11, P12, P13, P14). The substitutions of G by T at position 462 on the *matK* gene fragment and T by C at position 472 on ITS-rDNA could be considered as genetic diversity in different distribution populations.

Table 2. Comparison of ITS-rDNA gene sequence among the samples and eight taxa of *Panax* genus in Vietnam

Taxon	Locus																																											
	1 6	3 7	4 5	5 9	6 2	7 0	7 2	8 5	8 5	1 0	1 6	1 7	1 8	1 9	1 8	1 7	2 0	2 7	3 1	3 8	3 6	4 0	4 8	4 0	4 1	4 2	4 3	4 4	4 6	4 7	4 8	5 1	5 4	5 3	5 6	5 6	5 9	5 9	6 0					
P. stipuleanatus (1)	C	C	C	C	A	C	A	A	T	A	T	T	C	C	A	T	G	A	C	G	C	C	G	C	A	T	G	T	C	T	C	T	A	C	A	G	C	T	A	C				
P. stipuleanatus (2)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
<i>Panax</i> sp.1 (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Panax</i> sp.1 (4)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Panax</i> sp.2 (5)	.	.	T	.	G	.	.	G	C	G	G	C	T	.	T	C	A	G	.	A	T	A	.	.	C	.	.	T	.	T	A	.	A	.	A	.	.	C	G	.	.			
P. vietnamensis (6)	T	.	T	.	G	.	.	G	C	G	G	C	T	.	T	C	A	.	.	T	A	T	.	.	C	.	.	.	.	T	A	.	.	G	A	.	C	G	T	.	.			
P. vietnamensis var. fuscidiscus (7)	.	.	T	.	G	.	.	G	C	G	G	C	T	.	T	C	A	G	.	A	T	A	.	.	C	.	.	T	.	T	A	.	A	.	A	.	C	G	.	.				
P. notoginseng (8)	.	A	T	T	G	T	G	G	C	G	G	C	.	T	.	C	A	.	T	.	A	T	T	G	C	T	A	.	T	A	T	.	G	A	T	C	G	.	.					
P. vietnamensis var. langbianensis (9)	.	.	T	.	G	.	.	G	C	G	G	C	T	.	T	C	A	.	.	A	T	A	.	.	C	.	.	.	.	T	A	.	A	G	A	.	C	G	T	.	.			
P. bipinnatifidus (10)	.	.	T	.	G	.	.	G	C	G	G	C	T	.	C	A	.	.	A	.	A	T	.	G	C	.	.	.	.	T	A	.	.	G	A	.	C	G	.	.				

**Noted:** Studied samples and genbank accession numbers (1): P15, P19; (2): P16-P18; (3): P09, P14; (4): P10-P13; (5): P25, P26; (6): P01-P08; (7): P20-P24; (8): P27; (9): KX768322 (*N.V.Duy et al., 2016*); (10): HQ112362, HQ112417, HQ112456 (*Zuo Y. et al., 2011*).

3.2. Phylogenetic analysis

The ML analyses for individual DNA regions were mostly congruent, but relationships among major clades were poorly resolved. So, the combined ITS-rDNA and *matK* data set retrieved a well-supported topology for *Panax*. Topology of the BI analysis was mostly congruent with the ML tree. Thus, the ML tree with bootstrap (BS) and Bayesian posterior probability (PP) values is presented in Figure 2. Our phylogenetic analyses recognized five well-supported clades in *Panax*. The two remaining taxa (*Aralia*) grouped in another well-supported lineage (BS = 93%, PP = 1.00; Figure 2).

The phylogeny constructed from combined ITS-rDNA and *matK* data showed that Tam that

hoang (*P. stipuleanatus*) and Tam that la xe (*Panax* sp.1) are 100% identical and belong to the same clades. Otherwise, the samples of them separated into two clades related to geographical isolation. Clade II.1 including samples collected in Ha Giang with ML bootstrap and Bayesian PP values are 96% and 0,93; Clade II.2 including samples collected in Lao Cai with ML bootstrap and Bayesian PP values are 100% and 1,00 respectively. The results of our recent morphological study also showed that Tam that hoang (*P. stipuleanatus*) and Tam that la xe (*Panax* sp.1) are similar in rhizomes, flowers, fruits, and seeds; they are only distinguished in leaflets shape (entire leaflets in Tam that hoang and pinnate-leaflets in Tam that la xe) [18].

Table 3. Comparison of *matK* gene sequence among the samples and eight taxa of *Panax* genus in Vietnam

Taxon	Locus																											
	1 0 7	1 0 8	1 9 6	2 1 6	2 1 7	2 2 6	2 5 6	3 0 0	4 3 1	4 6 2	4 6 6	4 7 3	5 6 1	6 3 5	7 0 3	7 1 2	7 2 8	8 3 0	8 4 2	9 0 3	9 1 4	9 4 4	1 0 4	1 0 7	1 0 8	1 0 9		
<i>P. stipuleanatus</i> (1)	C	T	T	A	A	A	A	T	A	T	T	C	T	T	A	A	C	T	C	C	A	A	A	T	T	A	T	
<i>P. stipuleanatus</i> (2)	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Panax</i> sp.1 (3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Panax</i> sp.1 (4)	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>Panax</i> sp.2 (5)	A	G	A	G	G	C	G	G	C	G	C	.	C	G	.	T	T	G	.	A	.	C	G	G	G	C	.	
<i>P. vietnamensis</i> (6)	A	G	A	G	G	C	G	G	C	G	C	.	C	G	.	T	T	G	.	A	G	C	G	G	G	C	.	
<i>P. vietnamensis</i> var. <i>fuscidiscus</i> (7)	A	G	A	G	G	C	G	G	C	G	C	.	C	G	.	T	T	G	.	A	.	C	G	G	G	C	.	
<i>P. notoginseng</i> (8)	A	G	A	.	.	C	.	G	C	G	C	.	C	G	C	T	T	G	A	A	.	C	G	G	G	C	.	
<i>P. vietnamensis</i> var. <i>langbianensis</i> (9)	A	G	A	G	G	C	G	G	C	G	C	.	C	G	.	T	T	G	.	A	G	C	G	G	G	C	A	
<i>P. bipinnatifidus</i> (10)	A	G	A	G	G	C	G	G	C	G	.	.	C	G	.	T	T	G	.	A	.	C	G	G	G	C	.	

**Noted:** Studied samples and genbank accession numbers (1): P15, P19; (2): P16-P18; (3): P09, P14; (4)P10-P13; (5): P25, P26; (6): P01-P08; (7): P20-P24; (8): P27; (9): KX768328 (*N.V.Duy et al., 2016*); (10): HQ113000, HQ113055, HQ113094 (*Zuo Y. et al., 2011*).

Therefore, by the analysis of ITS-rDNA and *matK* genes sequences and phylogenetic analysis of these two gene regions, we suggest that Tam that la xe and Tam that hoang are belong to the same species as *Panax stipuleanatus* H.T.Tsai et K.M.Feng. However, to determine whether Tam that la xe is a variety of *P. stipuleanatus*, it is necessary to carry out more studies on others molecular markers or even chemical composition.

Comparison between the samples of Sam puxailaileng with Sam lai chau (*P. vietnamensis* var. *fuscidiscus*) found that ITS-rDNA and *matK* gene sequences of them are 100% identical. The phylogenetic analysis on ML tree also showed the dissociation of Sam puxailaileng along with Sam lai chau are well-supported lineage (BS = 97% and PP = 1,00). On the other hand, in morphology, two Sam puxailaileng samples have identical characters with Sam lai chau such as: Rhizome horizontal with alternate scars, leaflet unlobed, without stipules, disk flower flat (Figure 1-F,G,H).

Thus, by the analysis of ITS-rDNA and *matK* gene sequences and phylogenetic analysis

based on these two gene fragments, we suggest to identify Sam puxailaileng samples (P25, P26) collected at Pu Xai Lai Leng mountain (Ky Son district, Nghe An province) as *P. vietnamensis* var. *fuscidiscus* K.Komatsu, S.Zhu & S.Q.Cai. This results contribute a new location of *P. vietnamensis* var. *fuscidiscus* in Vietnam.

#### 4. Conclusions

Base on the combined ITS-rDNA and *matK* dataset, this study retrieved a well-resolved phylogeny of *Panax* species/varieties distributed in Vietnam. Whereby, the results suggest to identify Tam that la xe, which is used to confusing with Sam vu diep (*Panax bipinnatifidus* Seem.) as *Panax stipuleanatus* H.T.Tsai et K.M.Feng. In addition, this results also support Sam puxailaileng as *Panax vietnamensis* var. *fuscidiscus* contributing a new distribution point of this variety in Vietnam. The study also showed that ITS-rDNA and *matK* genes are high potential in distinguishing and identifying the taxa of *Panax* genus.

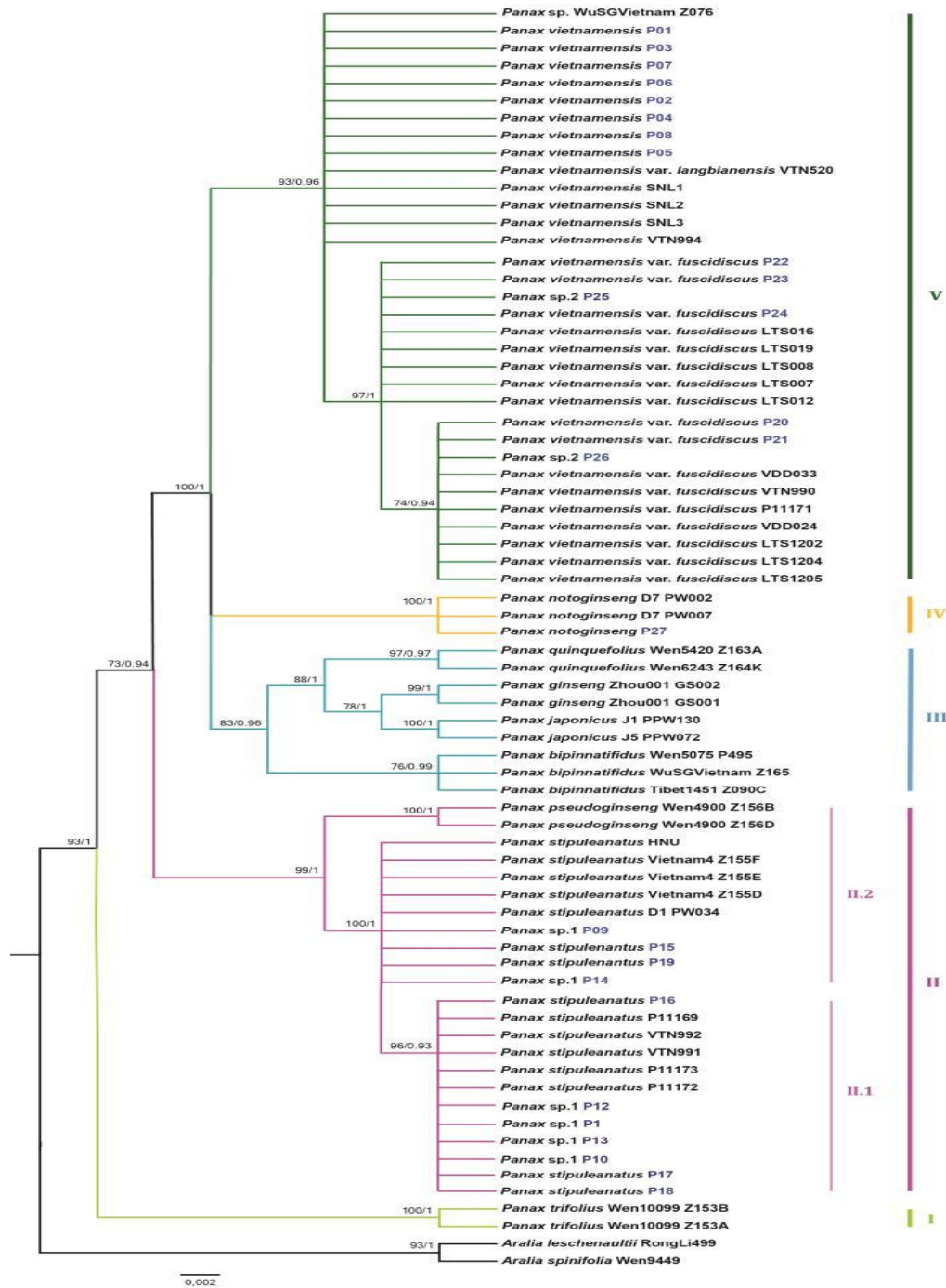


Figure 2. Maximum likelihood (ML) tree for *Panax* genus based on the combined ITS-rDNA and *matK* data (ML bootstrap (BS) and Bayesian (PP)-values) are indicated above branches as BS/PP form).



## Acknowledgements

The authors sincerely give many thanks to M.Sc. Master of Science Nguyen Xuan Nam and M.S. Pham Ngoc Khanh from National Institute of Medicinal Materials, and M.S. Phung Van Hao from Vinh University, who provided some of the samples in this study.

## References

- [1] Xiang, Q.B. & Lowry, P.P. Araliaceae, In: Wu, C.Y., Rawen, P.H. & Hong (2007).
- [2] Nguyen Tap, The species of *Panax* L. in Vietnam, Journal of Medicinal Material, 10 (3) (2005) 71-76 (in Vietnamese).
- [3] Phan Ke Long, Le Thanh Son, Phan Ke Loc, Vu Dinh Duy and Pham Van The, Lai Chau ginseng *Panax vietnamensis* var. *fuscidiscus* K. Komatsu, S. Zhu & S.Q. Cai. morphology, ecology, distribution and conservation status, Proceedings of the 2<sup>nd</sup> VAST – KAST workshop on biodiversity and bio-active compounds, Hanoi- Vietnam, October 28<sup>th</sup> – 29<sup>th</sup> (2013), pp. 65-73, Publishing house for science and technology.
- [4] Nong Van Duy, Le Ngoc Trieu, Nguyen Duy Chinh & Van Tien Tran, A new variety of *Panax* (Araliaceae) from Lam Vien Plateau, Vietnam and its molecular evidence, J. Phytotaxa 277(1) (2016) 047-058. <http://dx.doi.org/10.11646/phytotaxa.277.1.4>
- [5] Tran Ngoc Lan et al., Results of study on Sam Puxailaileng in high mountain, Nghe An province, Nghe An journal of Science and Technology 12 (2016) 7-11 (in Vietnamese).
- [6] Do Huy Bich et al., Medicinal plant and animal in Vietnam, ep. II (2004), Science & Technic Pub. pp. 1255 (in Vietnamese).
- [7] X. Chen, B. Liao, J. Song, X. Pang, J. Han, S. Chen, A fast SNP identification and analysis of intraspecific variation in the medicinal *Panax* species based on DNA barcoding, Gene 530(1) (2013) 39-43. <http://doi.org/10.1186/j.gene.2013.07.097>
- [8] Jun Wen and Elizabeth A. Zimmer, Phylogeny and Biogeography of *Panax* L. (the Ginseng Genus, Araliaceae): Inferences from ITS Sequences of Nuclear Ribosomal DNA, Molecular Phylogenetics and Evolution 6(2) (1996) 167-177.
- [9] C. Lee, J. Wen, Phylogeny of *Panax* using chloroplast trnC–trnD intergenic region and the utility of trnC–trnD in interspecific studies of plants, Mol Phylogenet Evol 31(2004): 894-903, DOI: 10.1016/j.ympev.2003.10.009
- [10] Y. Zuo, Z. Chen, K. Kondo, T. Funamoto, J. Wen, S. Zhou, DNA barcoding of *Panax* species, Planta Med. 2011 Jan, 77(2) (2011):182-187. Doi: 0.1055/s-0030-1250166.
- [11] K. Komatsu, S. Zhu, S.Q. Cai, A new variety of the genus *Panax* from Southern Yunnan, China and its nucleotide sequences of 18S ribosomal RNA gene and matK gene, J. Jap. Bot 78(2) (2003) 86-94.
- [12] V. Manzanilla, A. Kool, L. Nguyen Nhat, H. Nong Van, H. Le Thi Thu and H. J. de Boe, Phylogenomics and barcoding of *Panax*: toward the identification of ginseng species. BMC Evolutionary Biology (2018) 18-44. <http://doi.org/10.1186/s12862-018-1160-y>
- [13] Vu Huyen Trang, Hoang Dang Hieu, Chu Hoang Ha, Study on DNA barcode for identify Sam ngoc linh, National conference of biotechnology, Ep.2, (2013) 1100-1104.
- [14] Nguyen Thi Phuong Trang, Nguyen Thi Hong Mai, Zhuravlev Yury N, Reunova Galina D., rbcL and rpoB gene sequences of *Panax vietnamensis* var. *fuscidiscus* and *Panax vietnamensis*, the background for identification and comparison, Journal of Biology 39(1) (2016) 80-85. <http://doi.org/10.15625/0866-7160/v39n1.7870>
- [15] Phan Ke Long, Tran Thi Viet Thanh, Nguyen Thien Tao, Phan Ke Loc, Nguyen Tu Lenh, Nguyen Tien Lam, Dang Xuan Minh, Morphological and molecular characteristics of *Panax* sp. (Araliaceae) from Phu Xai Lai Leng mountain, Nghe An province, Vietnam, Journal of Biology 36(4) (2014) 494-499. <https://doi.org/10.15625/0866-7160/v36n4.5212>
- [16] Phan Ke Long, Vu Dinh Duy, Phan Ke Loc, Nguyen Giang Son, Nguyen Thi Phuong Trang, Le Thi Mai Linh, Le Thanh Son, Phylogenetic relationships of the *Panax* samples collected in Lai Chau province based on matK and ITS-rDNA sequences, Journal of Biology 12(2) (2014) 327-337 (in Vietnamese).
- [17] Nguyen Thi Phuong Trang, Le Thanh Son, Nguyen Giang Son, Phan Ke Long, A new ginseng species *Panax* sp. (Araliaceae) in Vietnam, Journal of Pharmacology 10 (2011) 59-63.
- [18] Pham Thi Ngoc, Pham Thanh Huyen, Nguyen Quynh Nga, Nguyen Minh Khoi, Morphological characteristics of genus *Panax* L. (Araliaceae) in Vietnam, Journal of Medicinal Materials 22(3) (2017) 315-322.