



Original Article

Taxillus Thibetensis (Lecomte) Danser (Loranthaceae) a New Record Species For Flora of Vietnam

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Abstract: *Taxillus thibetensis* (Lecomte) Danser (Loranthaceae), a species previously known only from China, is newly recorded from Vietnam. A specimen discovered in the rainforest of Lao Cai province, Northern Vietnam was identified as *Taxillus thibetensis* based on both morphological and molecular data. The Vietnamese individuals of *Taxillus thibetensis* are closely related to *T. thibetensis* in China. A detailed description, illustration and data on distribution, ecology, phenology of Vietnamese *Taxillus thibetensis* are provided.

Keywords: Loranthaceae, *Taxillus Thibetensis*, New Record, Morphology, Molecular Data.

1. Introduction

Taxillus Tiegh., the genus of subtribe Scurrulinae (Loranthaceae) includes ca. 35 species distributed from China to Southeast Asia and one species in Africa (coastal area of Kenya) [1-3]. Pham [4] recorded four species of *Taxillus* in Vietnam including: *T. balansae*, *T. delavayi*, *T. kwangtungensis*, *T. chinensis*. However, Nguyen [5] lumped members of *Taxillus* and *Scurrula* L. into the genus *Taxillus*, thus this genus includes

13 species in Vietnam. Le et al. [6] suggested to recognized *Scurrula* and *Taxillus* as two separate genera, and the genus *Taxillus* includes 5 species in Vietnam.

During the field trip in October 2019, the *Taxillus* specimens were collected in Ban Khoang commune, Sapa district, Lao Cai province of Vietnam. After analyzed the morphological characters of the specimens, we

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recognized the plant is *Taxillus thibetensis* (Lecomte) Danser, a species found only in China before. This is the first time *T. thibetensis* found in Vietnam and this newly recorded species updates the total number of *Taxillus* in Vietnam to six species. In this article, the identifying characteristics, pictures, and phylogenetic position, of *Taxillus thibetensis* are presented and discussed.

2. Materials and Methods

2.1. Taxon Sampling, DNA Extraction, Amplification, Sequencing

Studied specimens include dry specimens kept in the herbarium at Institute of Ecology and Biological Resource (HN), and department of Biology, Hanoi Pedagogical University No. 2, together with new specimens obtained during the survey in Lao Cai Province. Furthermore, the morphology of *Taxillus thibetensis* was also compared to other specimens in herbaria: PE (Beijing, China), KUN (Kunming, China). The herbarium codes follow the Index Herbariorum (<http://sweetgum.nybg.org/ih/>).

The descriptions from the Flora of China account [1] has been assessed and used as the basis for an expanded description of the species. Nomenclatural practice follows the International Code of Nomenclature for algae, fungi, and plants (ICN) [7].

For the molecular analyses, we assembled all sequences of *Taxillus* and *Scurrula* for five genes from Genbank (NCBI) including nuclear small-subunit ribosomal DNA (SSU rDNA), large-subunit ribosomal DNA (LSU rDNA), and three chloroplast DNA regions (*rbcL*, *matK* and *trnL-F*) (Table 1); the species *Dendrophthoe longituba* was selected as outgroup [3, 8-11]. Voucher information and GenBank accession numbers are listed in (Table S1). Moreover, we extracted genomic DNA and sequencing five gene regions of *Taxillus thibetensis* collected in Lao Cai, then added sequences of the *Taxillus thibetensis* to the datasets obtained from Genbank to construct the molecular phylogenetic trees. Genomic DNA was extracted from silica gel dried tissues following Doyle and Doyle [12].

Table 1. Primers used for PCR and sequencing in this study

Locus	Primer	Sequence 5'-3'	Reference
Chloroplast			
<i>matK</i>	78F	CAGGAGTATATTTATGCACT	Vidal-Russell & Nickrent [8,13]
	1420R	TCGAAGTATATACTTTATTCG	
<i>rbcL</i>	1F	ATGTCACCACAAACAGARAC	Vidal-Russell & Nickrent [8,13]
	889R	CTATCAATAACTGCATGCAT	
<i>trnL-F</i>	C	CGAAATCGGTAGACGCTACG	Taberlet et al. [14]
	F	ATTTGAACTGGTGACACGAG	
Nuclear			
LSUr DNA	27F	CCCGCTGAGTTTAAGCATA	Vidal-Russell & Nickrent [8,13]
	950F	GCTATCCTGAGGGAACTTC	
SSUr DNA	12F	TCCTGCCAGTASTCATATGC	Vidal-Russell & Nickrent [8,13]
	1796R	CACCTACGGAAACCTTGTT	

Polymerase chain reactions and sequencing were performed using the primers designed by Vidal-Russell & Nickrent [8,13] and Taberlet et al. [14]. The primers used for conducting PCR and sequencing were presented in Table 1. The PCR amplification reactions used MasterMix of

the BioMed company. The PCR program consisted of 5 min at 95°C, 36 cycles of 30s at 95°C, 50 s at 49°C, and 1 min 30s at 72°C, with a final extension of 10 min at 72°C.

PCR products were purified on 1.0% agarose gels. The all PCR products were purified using

BioMed multifunctional DNA fragment purification recovery kits then were sequenced using the amplification primers. The bidirectional sequencing was completed using the ABI 3730 DNA Sequencer (Applied Biosystems, Carlsbad, California, USA). The sequences were aligned in Geneious v.8.0.5 [15].

2.2. Phylogenetic Analyses

Both the maximum likelihood (ML) and Bayesian inference (BI) were carried out for the phylogenetic analyses.

The ML analysis was performed using the program RAxML 8.2.10 [16,17] with the GTR + I + G substitution model for each molecular marker and the combined dataset at the Cyber Infrastructure for Phylogenetic Research (CIPRES; www.phylo.org). ML bootstrap analysis was implemented with 1000 replicates. Bayesian inference was conducted in MrBayses 3.1.2 [18]. The best-fitting models for each marker and the combined data set were determined by the Akaike information Criterion (AIC) as implemented in jModelTest 2.1.6 [19]. Bayesian analysis of the combined data set used the GTR + I + G model as determined in jModelTest. The MCMC algorithm was run for 5,000,000 generations with four Markov chain Monte Carlo (MCMC) and trees were sampled every 1000 generations. The program Tracer 1.6 [20] was used to check that effective sample size (ESS) for all relevant parameters were well above 200 indicating that stationarity probably had been reached. With the first 25% of sampled generations (2500 trees) discarded as burn-in, a 50% majorily-rule consensus tree and posterior probabilities (PP) were obtained using the remaining trees.

3. Results and discussion

Our molecular analyses based on combined dataset from five makers strongly supported the new samples collected from Lao Cai province grouped with *Taxillus thibetensis* (the samples from China) with strong bootstrap support (BS:

100%, PP: 1.0) (Figure 1). Furthermore, by comparing specimens of *Taxillus*, especial type specimen of *Taxillus thibetensis* in the herbaria and the description in the Flora of China, we identified the collection as *T. thibetensis*, a species previously unrecorded from Vietnam.

The results from molecular analyses also suggest to recognize two separate genera *Taxillus* and *Scurrula* with strongly supported. The Vietnamese individuals of *Taxillus thibetensis* are closely related to *T. thibetensis* in China (Figure 1). *T. thibetensis* were characteristic by abaxial surface tomentose while adaxial surface rapidly glabrescent, corolla exterior pilose with dense verticillate hairs and tip of bud ellipsoid (Figure 2).

4. Taxonomic treatment

Describe a new record species for flora of Vietnam:

Taxillus thibetensis (Lecomte) Danser, Bull. Jard. Bot. Buitenzorg, sér. 3, 10: 355. 1929.

Type:—CHINA: Dêqên County, Tsekou, 15 June 1895, R. P. Soulié 1340 (Syntype!, P); Yunnan Prov., Dêqên County, Tsekou, *Monbeig s. n.* (Syntype, P).

Description: Aerial parasite, shrubs 0.8–1.2 m tall, young stems mostly tomentose, becoming glabrous when older, hairs yellowish brown, rarely white, both verticillate and stellate. Young branchlets tomentose with dense rusty red scales. Branches black or gray, almost glabrous, subsmooth, scattered lenticellate. Leaves opposite or subopposite; young leaf with dense rusty red scales, petiole 1 cm, pilose; leaf blade ovate or ovate-oblong, 6–11 × 3–5.5 cm, leathery, abaxial persistently tomentose, adaxial rapidly glabrescent, lateral veins 5–8 pairs, base subrounded, margin entire or undulate, apex obtuse or acute. Inflorescences axillary or at leafless node, umbels 2-3-fascicled, 3–5-flowered; peduncle and rachis 3–8 mm. Flowers bisexual, 4-merous, zygomorphic, yellow-brown or brown, rarely white, tomentose; bracts ovate, ca. 1 mm, apex acute.

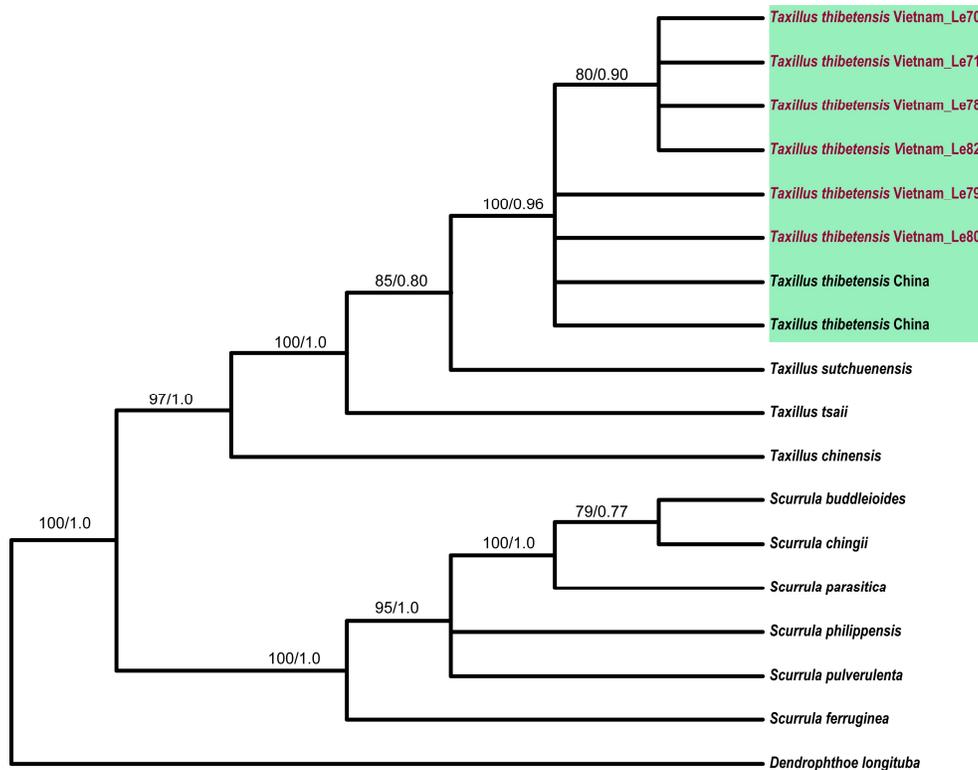


Figure 1. Majority rule consensus tree from a Bayesian analysis of the concatenated data set that includes the five genes. ML bootstrap values and posterior probabilities (PP) of the BI analysis are presented above the branches.

Pedicle 2–5 mm. Calyx ellipsoid, ca. 2 mm, limb annular, entire or minutely 4-toothed. Mature bud tubular 2.2–3.2 cm, tip ellipsoid. Corolla red, tube slightly curved, pilose with dense verticillate hairs, basal part inflated, lobes 4, lanceolate, 7–8 mm, split along one side at anthesis, reflexed. Stamens inserted at base of corolla lobes; filaments short, 1.5–2 mm; anthers 3.5–4 mm, multilocellate. Ovary 1-loculed; placentation basal. Style filiform, stigma capitate. Berry yellowish, ovoid or ellipsoid, 5–10 × 4–6 mm, granulose, pilose, with hairs, base tapering into stalk.

Habitat: Forests, mountain slopes, valleys, orchards, gardens; 2000-3000 m.

Phenology: Flowering in May–October; fruiting in August–November.

Distribution: New distributed points found in Vietnam are Lao Cai province. China

(Guizhou, SW Sichuan, SE Xizang, Yunnan).

Note: recorded hosts of *Taxillus thibetensis* including *Quercus* spp., *Prunus* spp. Kiu & Gilbert [1] suggested that *Castanea mollissima*, *Diospyros kaki*, *Pyrus pyrifolia*, and *Salix* spp. also recorded as hosts of *Taxillus thibetensis* in China.

IUCN Red List category: There have been no comprehensive field surveys of populations

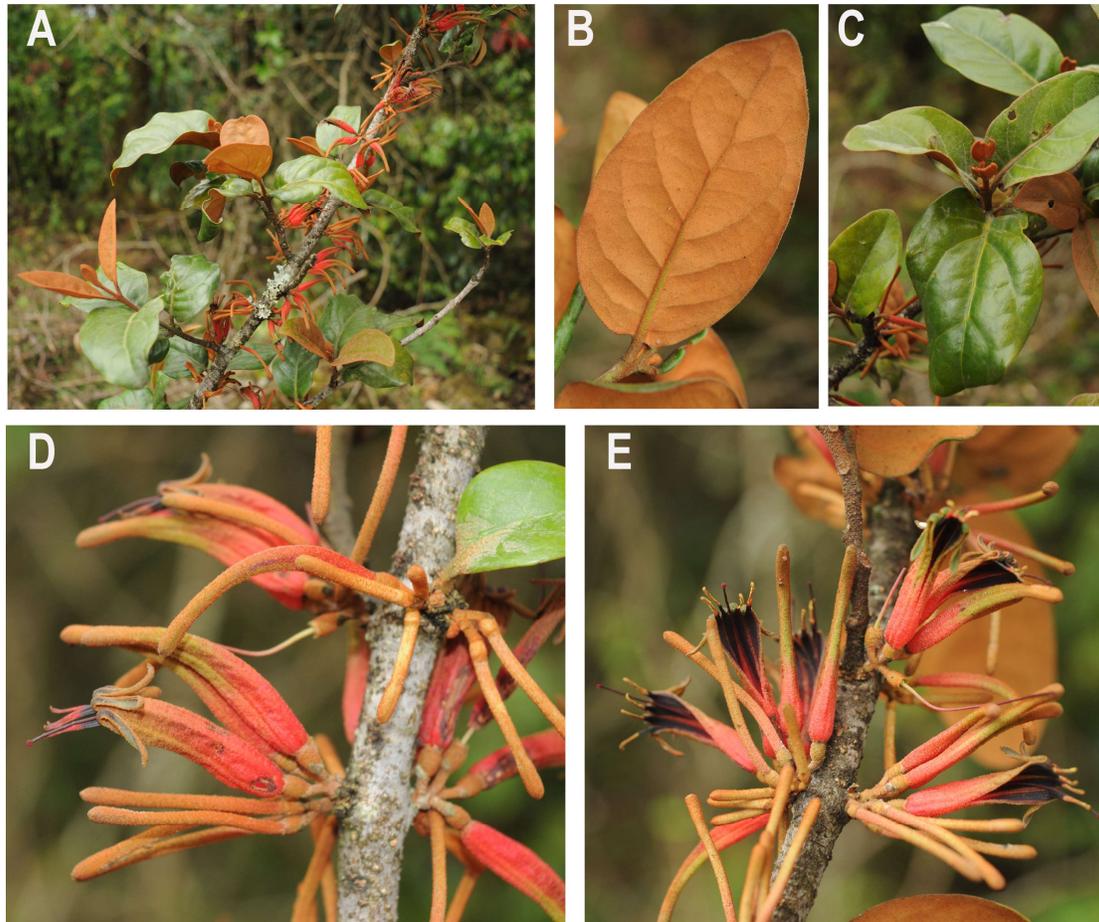


Figure 2. Morphology of *Taxillus thibetensis*. A: Habitat, Sapa, Lao Cai province, Vietnam. B: Abaxial and adaxial surfaces of leaf. C: Adaxial surfaces of leaf. D: Flower buds. E: Flowers.

of *Taxillus thibetensis*, so this species should be classified as Data Deficient (DD), according to IUCN Red List criteria (IUCN) [21]. Further field research may provide a more precise conservation assessment in the future.

Specimens examined: VIETNAM: Lao Cai prov. Sapa district, Ban Khoang commune, October 2019, *Van Du Nguyen, Hung Manh Nguyen, Xuan Thanh Trinh & Chi Toan Le* DMTT35, DMTT36, DMTT54, DMTT55,

DMTT58, DMTT62 (HN). CHINA: Yunnan Prov., Jingping County, Fenshuiling, 13 April 2015, *Bing Liu, Tianwen Xiao, Xiaoyang Yang 2894 & 2906* (PE).

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Table S1. Voucher information and GenBank accession numbers for DNA sequences generated or used in this study. The sequences generated in this study begin with MZ. “–” indicates missing data.

Species	Voucher/Source	Country of origin	LSU	SSU	mat K	rbcL	trnL-F
<i>Dendrophthoe longituba</i> (Elmer) Danser	D. L. Nickrent 4010 (SIU)	Malaysia	EU5 4436 6	–	EU5 4442 3	–	EU5 4448 5
<i>Scurrula buddleioides</i> (Desr.) G. Don	Z. J. Qiu 0096 (PE)	China	MG9 9940 2	MG9 9947 5	MG9 9942 3	MG9 9945 1	MG9 9949 9
<i>Scurrula chingii</i> (W.C. Cheng) H.S. Kiu	B. Liu 1736 (PE)	China	MG9 9940 3	MG9 9947 6	MG9 9942 4	MG9 9945 2	MG9 9950 0
<i>Scurrula ferruginea</i> (Jack) Danser	D. L. Nickrent 4008 (SIU)	Malaysia	EU5 4439 5	EU5 4434 3	EU5 4445 1	KF11 4863	EU5 4450 5
<i>Scurrula parasitica</i> L.	D. L. Nickrent 4004 (SIU); T. Yang BZXHDGK0047 (PE)	Malaysia, China	EU5 4439 7	EU5 4434 5	EU5 4445 1	MG9 9945 4	MG9 9950 2
<i>Scurrula philippensis</i> (Cham. & Schltdl.) G. Don	T. Yang BZXHDGK0054 (PE)	China	MG9 9940 5	MG9 9947 8	MG9 9942 6	MG9 9945 5	MG9 9950 3
<i>Scurrula pulverulenta</i> (Wall.) G. Don	M. Devkota 661 (KATH)	Nepal	EU5 4439 6	EU5 4434 4	EU5 4445 2	–	–
<i>Taxillus chinensis</i> (DC.) Danser	D. L. Nickrent 4032 (SIU); Z. D. Chen & C. T. Le 36 (PE)	Malaysia, Vietnam	EU5 4440 5	EU5 4435 0	EU5 4446 0	MG9 9945 6	MG9 9950 4
<i>Taxillus sutchuenensis</i> (Lecomte) Danser	Z. D. Chen 20010418 (PE)	China	MG9 9940 6	MG9 9947 9	MG9 9942 7	MG9 9945 7	MG9 9950 5
<i>Taxillus thibetensis</i> (Lecomte) Danser	C. T. Le et al. DMTT 35 (HN)	Vietnam	MZ4 2026 3	–	–	MZ4 2024 4	MZ4 2025 6
<i>Taxillus thibetensis</i> (Lecomte) Danser	C. T. Le et al. DMTT 36 (HN)	Vietnam	MZ4 2026 4	MZ4 2025 1	MZ4 2023 8	MZ4 2024 5	MZ4 2025 7
<i>Taxillus thibetensis</i> (Lecomte) Danser	C. T. Le et al. DMTT 54 (HN)	Vietnam	MZ4 2026 5	–	MZ4 2023 9	MZ4 2024 6	MZ4 2025 8
<i>Taxillus thibetensis</i> (Lecomte) Danser	C. T. Le et al. DMTT 55 (HN)	Vietnam	MZ4 2026 6	MZ4 2025 2	MZ4 2024 0	MZ4 2024 7	MZ4 2025 9
<i>Taxillus thibetensis</i> (Lecomte) Danser	C. T. Le et al. DMTT 58 (HN)	Vietnam	MZ4 2026 7	MZ4 2025 3	MZ4 2024 1	MZ4 2024 8	MZ4 2026 0
<i>Taxillus thibetensis</i> (Lecomte) Danser	C. T. Le et al. DMTT 62 (HN)	Vietnam	MZ4 2026 8	MZ4 2025 4	MZ4 2024 2	MZ4 2024 9	MZ4 2026 1

<i>Taxillus thibetensis</i> (Lecomte) Danser	<i>B. Liu 2894</i> (PE)	China	MG9 9940 7	MG9 9948 0	MG9 9942 8	MG9 9945 8	MG9 9950 6
<i>Taxillus thibetensis</i> (Lecomte) Danser	<i>B. Liu 2906</i> (PE)	China	MZ4 2026 9	MZ4 2025 5	MZ4 2024 3	MZ4 2025 0	MZ4 2026 2
<i>Taxillus tsaii</i> S.T. Chiu	<i>Z. D. Chen 20110126</i> (PE)	China	MG9 9940 8	MG9 9948 1	MG9 9942 9	MG9 9945 9	MG9 9950 7