

Regular Research Article

***Aquilaria yunnanensis* S.C. Huang (Thymelaeaceae), A New Record for the Flora of Vietnam**

Hoang Van Sam ^{1*}, Nguyen The Nha ¹, Tran Van Chu ¹, Nguyen Thanh Tuan ¹, Nguyen Thi Tho ¹,
Do Thanh Tam ¹, Le Bao Thanh ¹, Tran Ngoc Hai ¹, Ha Van Huan ¹, Hoang Thi Hang ¹, Duong Trung
Hieu ², Claudio Cerboncini ³, Olarte Alexandra ³

¹ Vietnam National University of Forestry, Hanoi, Vietnam

² North East College of Agriculture and Forestry, Quang Ninh, Vietnam

³ Institute for Bio- and Geoscience, Jülich institute, Germany

* Corresponding author: samhv@vnuf.edu.vn

Abstract: *Aquilaria yunnanensis* S.C. Huang (Thymelaeaceae), known to be endemic to Yunnan, is recorded for the first time from Dong Son Ky Thuong Nature Reserve, Quang Ninh Province, Vietnam. A taxonomic description and DNA analysis based on our Vietnamese collections are presented, together with information on its distribution, habitat and colour photographs.

Keywords: *Aquilaria*, Dong Son Ky Thuong Natural Reserve, Thymelaeaceae, Vietnam.

1. Introduction

Aquilaria Lam. (Thymelaeaceae) is a well-known important tree genus providing agarwood. It is endemic to the Indo-Malesia region, largely occurring from southern China throughout the Malesian region extending to the Pacific Islands (Domke, 1934), with the majority of species in Malesia (Hou, 1960). All species of the genus are currently protected under CITES regulation and are considered endangered on the IUCN Red List of Threatened Species (IUCN 2018). So far, 21 species have been recorded in the world, among which four occur in Vietnam, namely *A. crassna* Pierre ex Lecomte, *A. banaensae* Phamh., *A. baillonii* Pierre ex Lecomte and *A. rugosa* L.C. Kiet & Keßler (Ho 1992, Ho 2000, Kiet *et al.* 2005). *Aquilaria yunnanensis* was first described by S.C Huang in 1985, and this species has been supposed to be endemic in the Yunnan Province of China (Wang *et al.* 2007; Zhang *et al.* 2019) In 2017 and 2018, during field trips in Dong Son Ky Thuong Nature Reserve, Quang Ninh Province (Fig. 1), we collected specimens of this species. After making elaborate observations, DNA analysis and comparison with the specimens of the closely related species in herbarium VNF this species has been confirmed as *A. yunnanensis*.

2. Material and methods

Specimens were collected from 9 individual trees of *A. yunnanensis* in Dong Son Ky Thuong Nature Reserve, Quang Ninh Province. Specimens include branchlets, leaves, flowers and fruits. The collected specimens were re-examined using comparative methods (Thin 2007) and molecular analysis. Voucher specimens are deposited in herbarium VNF. Conservation status of plant species base on IUCN criteria 2014.

Total DNA was isolated from leaf samples of *Aquilaria* with the Plant DNA Isolation kit (Qiagen, Germany). Five samples collected from 5 individual samples in the study area were used for DNA analysis: DT04, DT06, DT09, HBQN01 and TDC01. Concentration, purity and integrity levels of genomic DNA were determined by spectrophotometry and electrophoresis. The nucleotide sequences of DNA fragments were processed and analysed by specialized software such as BioEdit, DNAClub in GenBank.

Table 1. List of primers to clone the fragments of DNA barcodes

Forward/Reverse primers	Primer sequence (dimensional 5'-3')	Cloned fragments of DNA barcode
mP1F	5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3'	<i>matK</i>
mP1R	5'-CGTACAGTACTTTTGTGTTTACGAG-3'	
rp1F	5'-ATGTCACCACAAACAGAGACTAAAGC-3'	<i>rbcL</i>
rp1R	5'-GTAATAATCAAGTCCACCRCG-3'	
trnPF1	5'-GTTATGCATGAACGTAATGCTC-3'	<i>trnH-psbA</i>
psbPR1	5'-CGCGCATGGTGGATTCACAATCC-3'	
IS2P1F	5'-ATGCGATACTTGGTGTGAAT-3'	<i>ITS</i>
IS2P1R	5'-TCCTCCGCTTATTGATATGC-3'	

3. Results

3.1 Taxonomic treatment:

Aquilaria yunnanensis S. C. Huang, in Acta Bot. Yunnan. 7: 277 (1985); Wang et al. in Wu, Z. Y., Raven, P. H. & Hong, D. Y. (eds.), in Flora of China 13: 214 (2007).

3.2 Vietnamese name

Dó vân nam.

3.3. Type

CHINA. Yunnan, Meng La, at 1200 m elev., 14 July 1963, Holotype - *Yan-hiu Li 4593* (KUN).

3.2 Morphological description

Medium trees, up to 15 m tall, diameter up to 40 cm. Branchlets dark brown, puberulous. Leaves simple, alternate; petiole 4–5 mm long, pubescent; Leaf blade elliptic-oblong or oblong-lanceolate, 7–10 × 2–4 cm, leathery, puberulous only on lower surface of the midrib, base cuneate or narrowly cuneate, apex caudate-acuminate 1.0–1.6 cm long, margin entire, upper surface dark green, smooth, lower surface light green; veins usually branched, dense, conspicuous and raised abaxially, obscure adaxially. Inflorescence terminal or axillary, consisting of 1, 2 or 3 umbels. Flowers fragrant; pedicel ca. 6 mm long, densely hairy. Calyx light yellow; tube campanulate, 6–8 mm long, exterior pubescent, interior 10-ribbed, ribs pubescent; lobes 5, ovate-oblong, ca. 3 mm long, densely pubescent both inside and outside. Petaloid appendages ca. 1.5 mm high, puberulous, apex rounded. Stamens 10, 1.5–2 mm long; anthers linear, introrse, ca. 1.0 mm long, filament pubescent, ca 1.0-1.3 mm long. Ovary subglobose, ca. 3 mm in diam, shiny, pubescent; style obscure; stigma capitate. Capsule obovoid, ca. 2.5 × 1.7 cm, base tapering and enclosed by erect, persistent calyx, corky when dried, apex rounded with apiculate tip; pericarp thickened, slightly rugose when dried, yellow pubescent. Fruit dehiscent with 2 valves stretched at acute angle. Seed(s) 1 or 2, ovoid, 6.5 -7.5 by 5.5 - 6.5 mm, densely brown-yellow pubescent, apex obtuse; funicle 0.8–1 cm long, slightly shorter or longer than seed.

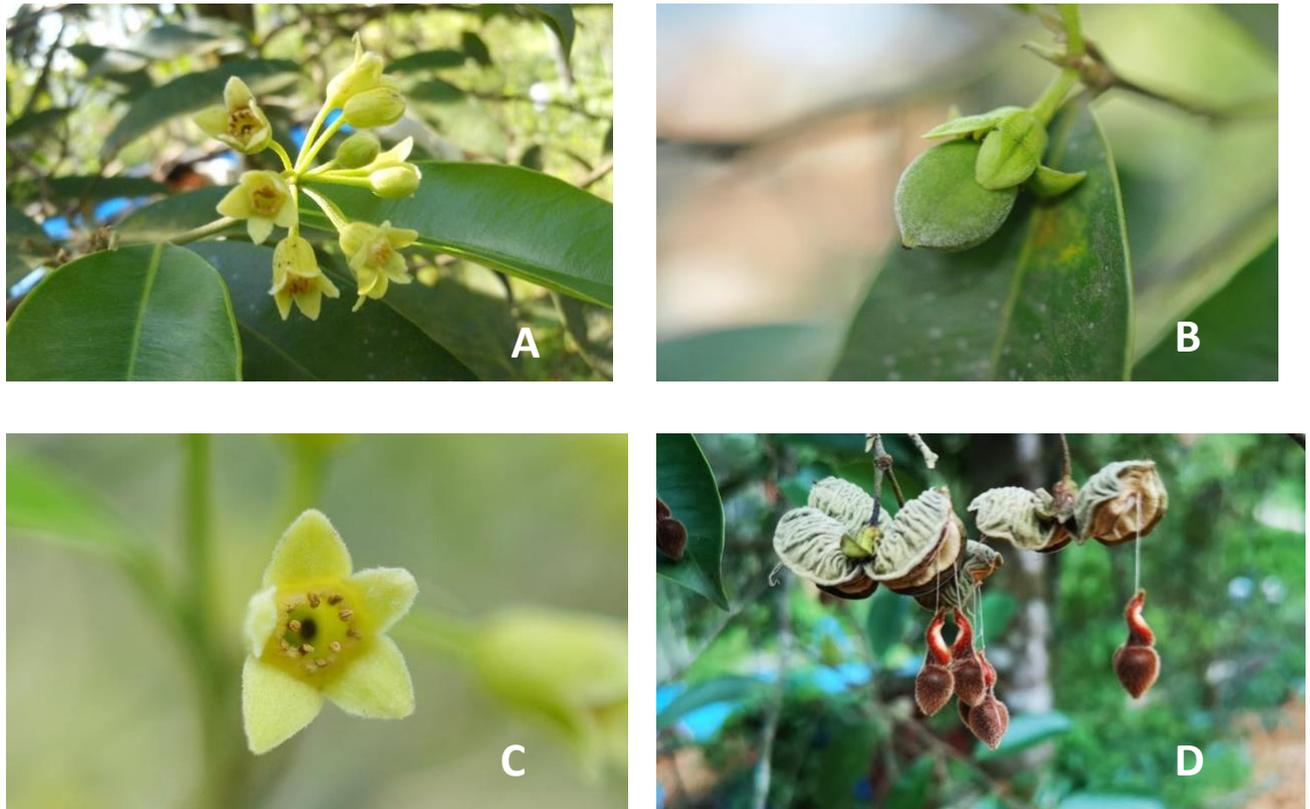


Figure 1. *Aquilaria yunnanensis* S. C. Huang: A. Branch with leaves and inflorescence; B. Fruit; C. Flower; D. Fruits open into 2 valves

3.3 Specimens examined

VIETNAM. Quang Ninh province, Hoang Bo district, Dong Son Ky Thuong Nature Reserve. Specimens in VNF; Nguyen DT04, specimen with fruits, 21.08'.147" N 107.05'.593"E, 28 June 2018, alt. 432 m; Nguyen DT06, specimen with fruits, 21.08'.172" N 107.05'.577"E, 28 June 2018, alt. 436 m; Nguyen DT09, specimen with fruits, 21.08'.186" N 107.05'.604" E, 28 June 2018, alt. 450 m; Nguyen & Hoang 1652017-01, specimen with flowers, 21°11'032"N 107°07'238"E, 16 May 2017, lt. 460 m; Nguyen & Hoang 1652017-02, specimen with flowers, 21°11'032"N 107°07'238"E, 16 May 2017, lt. 460 m; Duong HBQN01DT04, specimen with flowers, 21.08'.147" N 107.05'.593"E, 16 May 2017, lt. 432 m; Duong TDC01, specimen with flowers, 21.09'313" N 106.52.267" E, 16 May 2017, lt. 423 m.

3.4 Note

According to Wang *et al.* (2007), *A. yunnanensis* are small tree 3–8 m tall. However, in our research area we found 5 trees up to 15 m tall and 40 cm in diameter.

3.5 Distribution

China (Yunnan) and Vietnam (Dong Son Ky Thuong Nature Reserve, Quang Ninh Province).

3.4 Ecology

Aquilaria yunnanensis grows in natural forests in Dong So Ky Thuong Nature Reserve at altitudes from 400 to 480 m. In Yunnan, China, the species is distributed in ca. 1200 m Wang *et al.* (2007).

3.5 Conservation status

According to IUCN Red List 2019, *Aquilaria yunnanensis* is listed as Vulnerable (VU). Based on the result of our study, the number of mature individuals are only 9 in Dong Son Ky Thuong Nature Reserve, Quang Ninh province; this number is much smaller than criteria of IUCN (2014). (If any plant species has population smaller than 250 mature individuals, it should be listed as Critical Endangered (CR), additional also under threat from local people in the study area therefore , we propose this species should be listed as Critical Endangered (CR) in the Red Data Book and needs to be conserved in Vietnam. This species also should be upgraded to Endangered (EN) on the IUCN Red list.

3.6. Economic value

Similar to *Aquilaria crassa*, *A.yunnanensis* provides a high content and good quality resin (Hoang *et al.* in press), which is commonly used in the cosmetic industry, especially in producing perfume. Hence, this species should be considered as a high potential plant species for economic development of local people at least in the research area.

3.7 Phenology

Flowering from April to June and fruiting from June to August (based on the result of field observation).

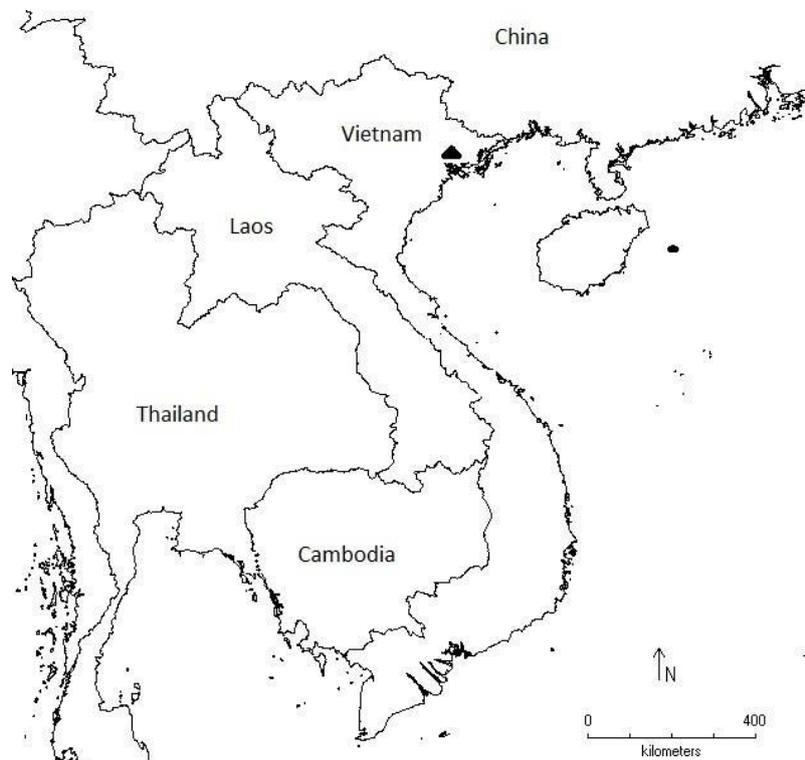


Figure 2. Map on the distribution of *A.yunnanensis* in Vietnam

3.8 GenBank accession no.:

KU244235.1 (*rbcl*) KY927340.1 (*matK*), KY927208.1 (*trnH-psbA*) and MH134148.1 (ITS2); we sequenced the specimen DT04, DT06, DT09, HBQN01 and TDC01

3.9 DNA analysis:

The results of sequence analyzing shown that *matK*, *rbcl*, *trnH-psbA* and ITS2 fragments with lengths of 852 bp, 588 bp, 441 bp and 393 bp respectively.

Table 2. Comparison of the nucleotide sequences of investigated samples of ITS2 locus with *Aquilaria yunnanensis* MH134148.1

Sample code	Positions			Differential nucleotides	Similarity (%)
	70	318	359		
DT04	C	C	C	0	100.00
DT06	C	C	C	0	100.00
DT09	T	T	T	3	99.24
HBQN01	C	C	C	0	100.00
TDC01	C	C	C	0	100.00
MH134148.1	C	C	C		

Results of comparing the sequence of 4 DNA fragments of 5 samples with *A. yunnanensis* (MH134148.1) in the NCBI Genbank show that there were no differences in the three DNA fragments (*matK*, *rbcl*, *trnH-psbA*) for all samples. For locus ITS2, the DT04, DT06, HBQN01 and TDC01 were the same *A. yunnanensis* (MH134148.1), a small difference (0.76%) was found in the DT09 sample.

Table 3. Morphological comparison among *Aquilaria* species

Characters	<i>Aquilaria yunnanensis</i>	<i>Aquilaria cassna</i> ¹	<i>Aquilaria sinensis</i> ²	<i>Aquilaria rugosa</i> ³
Size	Medium trees, up to 15 m tall	Big trees, up to 30 m tall	Medium trees, up to 15 m tall	Small trees, up to 10 m tall
Leaf shape	Elliptic-oblong or oblong-lanceolate, rarely obovate	Elliptic	Orbicular, elliptic-oblong, obovate	Ovate to obovate
Capsule shape	Obovoid, slightly compressed	Round, obovoid	Ovoid	Globose or pyriform
Valves	Valves thin, stretched at acute angle	Valves medium	Valves medium	Valves thick, angle of dehiscence to 180°
Surface of Capsule	Slightly rugose	Heavily wrinkled	White silky or smooth	Rugose

Pericarp	Pericarp thick and slightly rugose when dry	Pericarp thick, smooth when dry	Pericarp thin, smooth when dry	Pericarp thick and rugose when dry
Seeds	Seed(s) 1 or 2, densely brown-yellow pubescent	Seed(s) 2, black, glabrous	Seeds 2 white sericeous or glabrous	Seeds 2 dark-brown, densely covered with brownish hairs
Funicle	Funicle slightly shorter than seed	Funicle longer than seed	Funicle longer than seed	Funicle as long as seed

¹ Ho 1992, field observation and specimens in VNF (Nguyen Thanh Tuan QN04; Nguyen The Nha QN01; Nguyen The Nha PQ08; Nguyen The Nha PQ02); ² Wang et al. 2007; ³ Kiet et al. 2005, field observation and specimens in VNF (Phan Duc Le SN02; Nguyen The Nha ST02).

4. Conclusion

Our study confirms that Vietnam is home to five *Aquilaria* species, including *A. yunnanensis*. The study also provides useful information for the conservation of the important *Aquilaria* species in Vietnam as well as in the region.

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