

## A NEW SPECIES OF *AQUILARIA* (THYMELAEACEAE) FROM VIETNAM

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### SUMMARY

A new species of *Aquilaria* is described from Vietnam and keys to the flowering and fruiting specimens of all species from this region are provided. DNA sequences of the nrITS1-5.8S-ITS2 region seem to confirm its status as a distinct species.

**Key words:** *Aquilaria*, Thymelaeaceae, nrITS, taxonomy, Vietnam.

### INTRODUCTION

During a survey on wild and cultivated *Aquilaria* populations in the Central Highlands of Vietnam, one interesting *Aquilaria* was identified in the Sa Thay district, Kontum province. It is similar to other *Aquilaria* species in almost all vegetative characters, but critical examination of the fruits revealed that it is not only different from the three *Aquilaria* species so far described from Vietnam, namely *A. crassna* Pierre ex Lecomte, *A. baillonii* Pierre ex Lecomte, and *A. banaensis* P.H. Hô (Hô, 1992), but also to the whole genus *Aquilaria*.

This genus comprises so far 20 species, distributed in tropical and subtropical Asia from Southern China throughout the Malesian region extending to the Pacific Islands (Domke, 1934), with the majority of species in Malesia (Hou, 1960).

To check whether molecular characters would support recognition of the above described specimens as a distinct species, DNA sequences were obtained from the nuclear ribosomal ITS1-5.8S-ITS2 region, which was found to show variation between closely related species of *Aquilaria* and *Gyrinops* in a pilot study on molecular identification of cultivated and wild gaharu (Eurlings & Gravendeel, in press).

### MATERIALS AND METHODS

Total genomic DNA was extracted from 20 mg of silicagel dried leaf material of several different accessions of *Aquilaria* and *Gyrinops* (see Table 1) using the DNeasy Plant extraction kit and protocols of QIAGEN (Venlo, The Netherlands). PCR products of the nrITS1-5.8S-ITS2 region were collected using the primers described in White et al.

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Table 1. List of accessions used for DNA sequencing. Voucher specimens are deposited at the Leiden branch of the Nationaal Herbarium Nederland (L).

Species	Voucher	Year of Collection	Geographic origin	Genbank Accession Nr
<i>Aquilaria crassna</i> Pierre ex Lecomte	<i>Eurlings 1</i>	2004	Vietnam, An Giang province, Mt Giai	AY920326
<i>Aquilaria crassna</i> Pierre ex Lecomte	<i>Eurlings 7</i>	2004	Vietnam, An Giang province, Mt Giai, cultivated	AY920327
<i>Aquilaria rugosa</i> L.C. Kiet & Kiebler	<i>Heuveling van Beek 1</i>	2004	Vietnam, Kon Tum province	AY920328
<i>Aquilaria rugosa</i> L.C. Kiet & Kiebler	<i>Kiet 1940</i>	2004	Vietnam, Kon Tum province, cultivated	AY920329
<i>Aquilaria rugosa</i> L.C. Kiet & Kiebler	<i>Kiet 1941</i>	2004	Vietnam, Kon Tum province, cultivated	AY920330
<i>Gyrinops ledermannii</i> Domke	<i>Gunn 3030</i>	2004	Papua New Guinea, Yapsiei	AY920331

Table 2. Variable nucleotide positions of nrITS1, 5.8S and ITS2 regions in the six accessions analysed. Species-specific mutations for *A. rugosa* are indicated in dark grey, mutations shared with *G. ledermannii* are indicated in light grey.

	96	99	107	110	129	154	182	190	191	196	199	213	226	233	363	424	425	449	458	477	530	571	590	608	610	625	
<i>Aquilaria crassna</i>	T	C	C	T	C	C	C	T	C	A	C	G	G	C	C	T	C	G	C	A	A	C	A	C	C	G	G
<i>Aquilaria crassna</i>	T	C	C	T	C	C	C	T	C	A	C	G	G	C	C	T	C	G	C	A	A	C	A	C	C	G	G
<i>Aquilaria rugosa</i>	C	T	C	C	T	T	T	C	C	A	C	T	G	C	C	T	G	C	C	A	G	T	G	G	C	A	G
<i>Aquilaria rugosa</i>	C	T	C	C	T	T	T	C	C	A	C	T	G	C	C	T	G	C	C	A	G	T	G	C	C	A	G
<i>Aquilaria rugosa</i>	C	T	C	C	T	T	T	C	C	A	C	T	G	C	C	T	G	C	C	A	G	T	G	C	C	A	G
<i>Gyrinops ledermannii</i>	C	C	T	T	C	T	T	C	T	T	T	G	A	T	T	C	G	T	T	G	G	C	G	T	G	A	A

(1990). DNA sequences were obtained on an ABI 377 automated sequencer (Applied Biosystems) using the protocols described in Eurlings & Gravendeel (in press) and submitted to GenBank under accession numbers AY920326–AY920331.

## RESULTS

The nrITS1-5.8S-ITS2 alignment contained 649 positions of which 26 were variable. Five substitutions were unique to *A. rugosa*. Pairwise similarity between the latter species and *A. crassna* and *G. ledermannii* was 97.8% and 97.1%, respectively. Pairwise similarity between *A. crassna* and *G. ledermannii* was 96.7%. Furthermore, *A. rugosa* shared 13 mutations with *A. crassna* and eight mutations with *G. ledermannii* (see Table 2).

## DISCUSSION

The molecular results obtained seem to confirm the status of *A. rugosa* as a separate species, as a total of eight substitutions in the nrITS1-5.8S-ITS2 region were found between this species and the morphologically most similar *A. crassna* also occurring in Vietnam. It must be stressed, though, that more different accessions of all species of *Aquilaria* occurring in Vietnam need to be analysed to assess genotypic variability properly.

## TAXONOMIC TREATMENT

*Aquilaria* Lam. (1783) 49. — Type: *Aquilaria malaccensis* Lam.

Trees and shrubs. Inner bark without glistening fibres. Leaves alternate, entire; veins  $\pm$  parallel; margin thickened, recurved. Inflorescences axillary, supra-axillary, terminal or rarely born on the tree trunk or older branches, sessile or on short peduncles, simple or rarely branched, umbellate or paniculate. Flowers 5-merous, pedicelled, hypanthium (calyx tube or floral tube) cup-shaped, cylindrical or bell-shaped; calyx lobes (4 or) 5 (or 6), erect or recurved, usually shorter than calyx tube; petaloid appendages distinct, twice as many as calyx lobes, hairy, inserted at the base of calyx lobes; stamens 10, inserted in two whorls, anthers dorsifixed; disk none or rarely ring-like; ovary ovoid to ellipsoid, sessile or stiped, pubescent, 1- or 2-celled, ovule 1 per cell; style obscure or absent; stigma capitate or  $\pm$  globose. Fruits loculicidal capsules, ovoid, ellipsoid, oblanceolate or globose,  $\pm$  compressed, smooth or rugose; pericarp woody or spongy, dehiscent, valves stretched at acute to obtuse angles; seed(s) 1 or 2, ovoid or ellipsoid, apex acute, acuminate or beaked, base adnate to the caruncle-like or tail-shaped appendage and dangling at the ends of filiform or obscure funicles.

Distribution — About 20 species from India, Burma, Indochina, SE China, and widespread in Southeast Asia. In Vietnam so far 4 species recorded.

## KEYS TO AQUILARIA SPECIES IN VIETNAM

Flowering material (except *A. banaensis* of which flowers are unknown)

- 1a. Stigma capitate. . . . . *A. crassna*
- b. Stigma lobed . . . . . 2
- 2a. Stigma irregularly lobed, ovary rectangular in cross section . . . . . *A. rugosa*
- b. Stigma regularly lobed, ovary circular in cross section. . . . . *A. baillonii*

Fruiting material (except *A. baillonii* of which fruits are unknown)

- 1a. Exocarp smooth, capsules flattened, spindle-like . . . . . *A. banaensis*
- b. Exocarp slightly or heavily rugose, capsules obovoid or globose. . . . . 2
- 2a. Capsules obovoid, 4 by 3 cm, exocarp slightly rugose . . . . . *A. crassna*
- b. Capsules globose, 2 by 2 cm, exocarp heavily rugose . . . . . *A. rugosa*

***Aquilaria rugosa*** L.C. Kiet & Keßler, *spec. nov.* — Fig. 1

Arbuscula usque ad 10 m alta. Folia obovata vel obovata, 8–10(–12) cm longa, 3.5–4.5 cm lata, chartacea vel subcoriacea, apice caudato-acuminata, caudis usque ad 1 cm longis, nervis 20–25, ascendentis ad marginem incrassatum. Umbellae subsessiliae, pedicellis setosis. Hypanthium tubulare, infundibulare vel campanulatum, 4.5–6.5 cm longum, villosum, 10-costatum. Calyx 5 lobis, ovatis ad deltoideis, patentis vel reflexis, villosis tubo aequilongis. Appendices corollarum 10, ovatae vel semi-orbiculatae, ca. 1 mm longae, villosae. Stamina 10, 2-verticillata, glabra, antherae introrsae, ca. 1 mm longae. Ovaria 2.5–3.5 mm longa, in sectione transversali rectangularia, bilocularia, bilocularia, ovulum 1 per loculum. Stigma irregulare lobata. Capsula bilocularis bivalves loculicide dehiscens globosa vel pyriformis, apice truncato vel depresso, basin constricto, exocarpio rugoso, stipite 2–3 mm longo, hypanthio persistenti. Valvae maturae 2, expansae. Semina 2, 7.5 cm × 6.5 mm, tomentosa, apice acuto, caruncula tortili in sicco. — Typus: *Kiet 1936* (holo L), Vietnam, Kontum province, 500 m alt., in fruit, 17 July 2004.

*Tree* up to 10 m high, bole straight up to 3 m, or shrub 4–5 m. Bark grey, rugose with a fine network of polygonal fissures; woody twigs with reddish brown bark, longitudinally fissured, glabrescent; young twigs densely pubescent. Foliar buds covered with silky hairs. *Leaves* ovate to obovate, 8–10(–12) by 3.5–4.5 cm, chartaceous to subcoriaceous, dull, base attenuate or obtuse, rarely rounded, margin slightly recurved, white ciliate; apex shortly acute to acuminate, the acumen up to 1 cm long, both surfaces mostly glabrous, midrib sparsely appressed hairy beneath, depressed above, veins 20–25 pairs, subparallel, distinct beneath, obscure above, simple or sometimes forked, slightly curved and ascending towards the thickened marginal vein. Petiole 5–7 mm long, pubescent when young, canaliculated above. *Inflorescences* terminal on young twigs, or on short lateral branchlets, umbelliform, in 1-, 2- or many- (8–15-) flowered umbels, or sometimes branched with 2 or 3 umbels and each with 8–15 flowers, more rarely a simple umbel; umbel with a short peduncle or subsessile, densely hairy, usually with a few small (caducous) bracts; pedicels 0.8–1 cm, setose. *Hypanthium* (floral tube) whitish to yellowish, shortly tubular, funnel- or bell-shaped, 4.5–6.5 mm long, villose on both surfaces, distinctly 10-ribbed outside; calyx lobes 5, slightly ovate to deltoid, spreading or reflexed, villose, as long as the floral tube. *Petaloid appendages*



Fig. 1. *Aquilaria rugosa* L. C. Kiet & Keßler. a. Habit (*Heuveling van Beek 1*); b. flower bud; c. flower bud from above; d. flower bud at the beginning of anthesis; e. open flower, lateral view; f. open flower from above; g. flower (longitudinal section); h, i. stamens; j. closed capsule (*Kiet 1936*); k. capsule at early state with dangling seed; l. fully open capsule at maturity.

ovate or semi-orbicular, c. 1 mm long, densely villous, inserted slightly behind the stamens, free, about half-shorter than the stamens. *Stamens* 10, inserted in two whorls, slightly below the petaloid appendages, all with fleshy filaments, glabrous, the epise-palous ones longer; anthers oblong, introrse, c. 1 mm long, glabrous. Pistil c. 2 mm long, hairy. *Ovary* rectangular in section, 2.5–3.5 mm long, villose, 2-celled; 1 ovule per cell, hanging near the top of the ovary; style obscure or absent; stigma irregularly lobed, light brown. *Infructescences* terminal, umbelliform, sessile or on 1–2 mm long peduncles bearing 5–8 fruits. Pedicels c. 10 mm long. *Capsules* globose or pyriform, ash grey when dry, 2 by 2 cm, 2-celled, truncate to depressed at the apex, constricted at the base into a 2–3 mm long stipe, hypanthium (floral tube) persistent, campanulate, entire, c. 11 mm long, distinctly 10-ribbed outside, transverse section of capsule c. 2 cm across, marked by 4 lines, of which 2 prominent opposite ones belong to the suture; valves 2, thick, angle of dehiscence to 180° at the very maturity, mesocarp spongy when young; exocarp rugose (hence the name) outside at maturity or when dry, pericarp woody at maturity or when dry. *Seeds* 2, ovoid, dark-brown, 7.5 by 6.5 mm, acute at the apex; caruncle-like appendage as long as the seed, twisted when dry; seed and appendage densely covered with brownish hairs.

Distribution — Vietnam, Kontum, known only from the type locality.

Ecology — Recently cultivated at 500 m elevation.

Phenology — Flowering in April; fruiting in July.

Conservation status — *Aquilaria rugosa* is recorded from two home gardens where it was first cultivated using wild material collected from forest in the Sa Thay district 5 years ago. As the type locality is near the logged areas and the agarwood collectors are jealous to appropriate the wild trees for their own exploitation, we can consider that the species in its original habitat is endangered, although it is now successfully domesticated and produces agarwood by artificial inducement. For the moment it is known from few locations not far from areas prone to intensive and illegal logging, to which it seems restricted. It is likely that further inventory work in the forested areas of Sa Thay district will uncover new locations.

Notes — *Aquilaria rugosa* is similar in general appearance of vegetative habit and structures to most of the *Aquilaria* species, but it differs from the others by shape, ornamentation pattern of the epicarp surface, and dehiscence of the fruit. Rugose sculptures on the exocarp surface are rare in *Aquilaria*. When going through literature and the herbarium collections in the Herbarium Bogoriense, the Herbarium of Singapore and the Nationaal Herbarium Nederland, Universiteit Leiden branch, for the EC-TRP project (see acknowledgement) we came across all the species so far described in the literature of this genus. Three species have ornamented exocarp surface: *A. citrinacarpa* Hallier f. from the Philippines with small glandular-like dots; the Malesian species *A. microcarpa* Baill. with minute sinuous lines; only the Chinese species *A. yunnanensis* S.C. Huang has an exocarp surface similar to our *A. rugosa*, but the fruit shape is globoid and slightly compressed (vs globose-cordiform), with a convex apex (vs depressed apex), a dehiscence with 2 valves stretched at acute angle (vs 2 valves stretched at 180°), and the ovary is ovoid (vs shallow-longitudinally grooved).

The material used in this description consists of a specimen in flower collected in April 2003 by H. Heuveling van Beek and several specimens in fruit collected in July

2004 by the first author. Flowering and fruiting were profuse during our inventory activities. The wounded wood produces agarwood under natural traumatism and artificial stimulation.

Specimens examined:

VIETNAM. Kontum province; *Kiet 1936* (holotype L), *1940, 1941, Heuveling van Beek 1* (all L).

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