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RESEARCH ARTICLE

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Rediscovery of *Aquilaria rostrata* (*Thymelaeaceae*), a species thought to be extinct, and notes on *Aquilaria* conservation in Peninsular Malaysia

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Key words

Aquilaria
conservation status
molecular identification
Peninsular Malaysia
single mutations
taxonomy

Abstract After more than 100 years since the first discovery, *Aquilaria rostrata*, a critically endangered species listed in the IUCN Red List and presumably extinct, has been rediscovered in Terengganu State of Peninsular Malaysia. Here, we describe the history, taxonomy, ecology and conservation status of this endemic species, and compare our findings with the species description made from the first and only collection produced prior to this study. In addition, we present the similarities between *A. rostrata* and several *Aquilaria* species occurring in Peninsular Malaysia and neighbouring regions using molecular sequence data from the nuclear ribosomal DNA (Internal Transcribed Spacer) and chloroplast intergenic spacer region (*trn*L-*trn*F). Our morphological and sequence analyses support the separate status of *A. rostrata*, a long-lost endemic species of Malaysia.

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INTRODUCTION

Aquilaria is a genus of about 20 species (IPNI 2015), distributed mainly in the Indo-Malesian region, although the actual number of recognized species in the genus is still a subject of debate. Part of the disagreements over species number and application of correct names is caused by the difficulty of accurate identification, which relies chiefly on the flower and fruit characteristics (Hou 1960, 1964, Whitmore 1973, Tawan 2004). Accurate identifications of *Aquilaria* species are often difficult or even impossible as they are trees that are rarely spotted with fruits and flowers in the wild. The flowering period does not follow an annual cycle (Ito & Honda 2005, Chua 2008), thus making identification efforts an even more challenging task.

During a forest excursion in a strictly managed sustainably harvested natural forest in Terengganu, Peninsular Malaysia, a population of an unknown *Aquilaria* species was encountered in one of the reserved forest compartments. In the field, the newly encountered population is seemingly identical to *A. malaccensis* Lam., at least from the vegetative appearance. However, tree size and several characteristics of the fruits are different from *A. malaccensis* and *A. hirta* Ridl., which are the two common species found in Terengganu. After comparison with relevant taxonomic treatments (Ridley 1924, Hou 1960, Whitmore 1973), and comparisons with dried herbarium specimens deposited in SING and K we conclude that this population of *Aquilaria* is *A. rostrata* Ridl., a critically endangered species of *Aquilaria* under the IUCN Red List (Lim 2012). This is a new record for *A. rostrata* in Terengganu, over 100 km away from where it was first reported.

In this paper, we attempt to clarify the identity and relationships of *A. rostrata* within the genus. Apart from providing a taxonomic evaluation of this newly rediscovered population of this rare species, we also utilized modern molecular techniques to aid in future species recognition of *Aquilaria* species occurring in Peninsular Malaysia. The nuclear ribosomal ITS (nrITS) region and the intergenic spacer region *trn*L-*trn*F are known to be strong and reliable molecular markers to support morphological identification in the genus *Aquilaria* (Eurlings & Gravendeel 2005, Kiet et al. 2005).

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MATERIALS AND METHODS

Specimen collection

For the newly discovered *A. rostrata*, two separate collections were carried out. The first was on 28 April 2015, when fresh leaf samples and herbarium specimens (FBL03001–FBL03005) were collected. Unfortunately, the reproductive parts were then absent. An additional collection was made on 8 June 2015 from a tree with reproductive parts present (SE2072). Vouchers FBL03001–FBL03003 are deposited at SING, while SE2072 is deposited at both SING and KEP (acronyms according to Thiers, continuously updated). In addition, all voucher specimens are kept as our own collection in our laboratory (Forest Biotech Laboratory – FBL), Faculty of Forestry, Universiti Putra Malaysia.

Plant materials

Fresh materials were used as much as possible for the molecular study to ensure that genomic DNA of good quality is obtained. Fresh specimens of *A. rostrata* were collected as described above, while samples of *A. hirta* and *A. malaccensis* were collected during previous field expeditions in the states of Terengganu and Pahang, respectively. These two species are widely available in the two states and their occurrences have been reported previously (FDPM 2005). For *A. crassna* Pierre ex Lecomte and *A. subintegra* Ding Hou fresh material was collected from plants cultivated in Forest Research Institute of Malaysia (FRIM) under an ex-situ conservation program. In the absence of fresh material for *A. beccariana* Tiegh. and *A. microcarpa* Baill. fragments of leaf samples were sought from SING and were taken from dried herbarium specimens. Similarly, a sample of the type of *A. rostrata* was also sought from SING.

Table 1 Details on the specimens used in this study from which the ITS and *trn*L-*trn*F sequences were derived.

Species name	Collector's name and collection number	Collection Date	Deposition of vouchers	Specimen origin	Material analyzed	GenBank accession no.	
						nrITS	*trn*L-*trn*F
Aquilaria rostrata[1]	Lee et al., FBL03001	28 Apr. 2015	FBL, SING	PM: Terengganu	Fertile	KT364482	KT364475
Aquilaria beccariana[2]	Corner EJH, SFN29381	5 May 1935	SING	PM: Johor	Sterile	KT364477	KT364470
Aquilaria crassna[3]	Mohamed R, FBL01012	24 June 2014	FBL	Vietnam	Fertile	KT364478	KT364471
Aquilaria hirta[1]	Lee et al., FBL02088	27 Apr. 2015	FBL	PM: Terengganu	Fertile	KT364479	KT364472
Aquilaria malaccensis[1]	Lee SY & Mohamed R, FBL02046	17 Oct. 2013	FBL	PM: Pahang	Fertile	KT364480	KT364473
Aquilaria microcarpa[2]	Ilias Pa'ie, S15838	3 Dec. 1961	SING	Sarawak	Sterile	KT364481	KT364474
Aquilaria subintegra[3]	Mohamed R, FBL01015	24 June 2014	FBL	Thailand	Fertile	KT364483	KT364476
Gonystylus bancanus[1]	Lee SY, FBL01013	7 Apr. 2015	FBL	PM: Selangor	Fertile	KT896549	KT896550

[1] Recent collection for this study.
[2] Fragments of dried herbarium specimens are used.
[3] Collected from cultivated trees at FRIM.
FBL = Forest Biotechnology Laboratory, Universiti Putra Malaysia; PM = Peninsular Malaysia.

To serve as an outgroup, fresh material of *Gonystylus bancanus* Miq. was collected at Ayer Hitam Forest Reserve, Selangor, Malaysia. Details on the specimens used in this study are summarized in Table 1.

DNA extraction, PCR and sequencing

For fresh leaves, genomic DNA was extracted from a total of 1 g fresh tissue using the DNeasy® Plant Mini Kit (Qiagen, USA), according to the manufacturer's protocol. For herbarium specimens, genomic DNA was extracted from 20 mg of the dried leaf tissue using the same extract kit based on a modified and optimized protocol suggested by Costa & Roberts (2014). The quantity and quality were determined using NanoPhotometer™ (IMPLEN, Germany). For PCR amplification, the nrITS region was amplified using the forward primer, ITS92, 5'AAGGTTTCCGTAGGTGAAC3' and reverse primer, ITS75, 5'TATGCTTAAACTCAGCGGG3' (Baldwin 1992); while the *trn*L-*trn*F region was amplified using the forward primer, e, 5'GGTTCAAGTCCCTCTATCCC3' and reverse primer, f, 5'ATTTGAACTGGTGACACGAG3' (Taberlet et al. 1991). The final reaction volume was 25 µL, containing 12.5 µL of 2× PCRBIO Taq Mix Red (PCRBiosystems, UK), 0.4 µM for both forward and reverse primers, and 15 ng genomic DNA template. A negative control (without DNA template) was included in each run to verify the absence of contamination. PCR amplifications were conducted using MyCycler™ Thermal Cycler (Bio-Rad, USA), programmed for 1 min at 95 °C; 40 cycles for 15 s at 95 °C, 15 s at T_a and 1 min at 72 °C, with a final 3 min extension at 72 °C (T_a: ITS 50°C; *trn*L-*trn*F 55 °C). Amplification products were separated using electrophoresis on 1 % agarose gels in 1× TAE buffer, stained with ethidium bromide and photographed under UV light. PCR products were sent for direct Sanger sequencing (1st Base Laboratory Sdn. Bhd, Malaysia) using an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, USA) from both ends.

Data analysis

Complementary strands of the sequenced region were assembled using Gene Runner v. 3.05 (Hastings Software Inc., USA) and manually trimmed to obtain a clean sequence. The obtained sequences were deposited in GenBank. A homology check was carried out using NCBI BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the presence of species with similar sequence combination. Nucleotide multiple alignments were conducted with ClustalW embedded in MEGA6 (Tamura et al. 2013). Genetic distances were generated using the Kimura 2-parameter model (Kimura 1980), with all gaps treated as missing (complete deletion option). Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster trees were constructed using MEGA6, with bootstrap consensus of 1 000 replicates.

Results

All fresh samples yielded genomic DNA of good quality and PCR amplifications were successful using the extracted DNA. DNA extraction from herbarium specimens proved to be more challenging. Although they yielded low amount of genomic DNA, PCR products were still amplifiable from *A. beccariana* and *A. microcarpa* and sufficed for use in sequencing. However, this was not the case with the type material of *A. rostrata*. We assume that its DNA might have been too much degraded due to the long storage period (Staats et al. 2011). In this study, we are only reporting sequences from the newly discovered specimens of *A. rostrata*.

Two morphological groups were observed within the newly discovered population of *A. rostrata*, one with elliptic leaves and the other with lanceolate leaves. To test their validity, the ITS and *trn*L-*trn*F sequences from representatives of each of the two recognized morphological groups (FBL03001 and FBL03002, respectively) were compared and were found identical. Further analysis was then carried out using only the sequence from the FBL03001 specimen.

The ITS sequence of *A. rostrata* consists of 682 nucleotides, of which 31 are variable when compared to the other six *Aquilaria* species that we have sequenced. There are six substitutions unique to *A. rostrata* (Table 2). Pairwise similarity for *A. rostrata* is highest (97.4 %) with *A. malaccensis*, followed by *A. beccariana* (97.7 %), *A. microcarpa* (98.0 %), *A. crassna* and *A. subintegra* (98.2 %) and *A. hirta* (98.5 %). The *trn*L-*trn*F sequence of *A. rostrata* consists of 468 nucleotides, of which, unlike the highly variable ITS, only ten are variable and four are unique substitutions (Table 3). Pairwise similarity for *trn*L-*trn*F for *A. rostrata* is highest (98.5 %) with *A. beccariana*, *A. malaccensis* and *A. microcarpa*; and 98.7 % when compared to *A. crassna*, *A. hirta* and *A. subintegra*. Upon searching for sequence homology through the NCBI Blast program, the *A. rostrata* sequences matched other *Aquilaria* sequences deposited in the GenBank at very high levels, 95–98 % for ITS and 97–99 % for *trn*L-*trn*F (Table 4). The UPGMA tree based on ITS places *A. rostrata* into the same branch as *A. crassna* and *A. subintegra*, separate from the two (Fig. 1), while the tree based on *trn*L-*trn*F singles out *A. rostrata* from the other *Aquilaria* accessions on an independent branch (Fig. 2).

Discussion

As described by Ridley (1924), *A. rostrata* is characterized by the long-beaked capsules; this characteristic is obvious from our specimens (Fig. 3). We observed a few differences in vegetative and fruit characteristics and assume these are phenotypic (Table 5). Furthermore, using molecular approaches, we demonstrate that *A. rostrata* is well separated from other *Aquilaria* species commonly found in Malaysia and neighbouring countries.

Table 2 Nucleotide variation in the nuclear ribosomal ITS region of *Aquilaria rostrata* compared to six different *Aquilaria* species. Species-specific mutations in *A. rostrata* are indicated in dark grey, while mutations shared with other *Aquilaria* species are indicated in light grey. GenBank accession numbers are as listed in Table 1.

	ITS1								5.8S			ITS2											
Nucleotide positions	37	57	106	135	164	195	200	202	203	221	223	224	243	283	352	386	433	436	460	469	479	541	558	570	573	582	601	621	624	633	636
Aquilaria rostrata	C	T	T	C	T	T	C	G	T	C	G	T	T	C	C	T	C	G	C	C	C	G	G	C	A	C	G	A	C	C	A
Aquilaria beccariana	T	T	T	C	C	T	T	G	C	C	G	T	T	C	A	C	G	G	G	T	G	A	C	T	A	G	G	A	C	C	G
Aquilaria crassna	T	T	T	T	C	T	T	G	T	C	G	T	C	C	C	G	C	C	G	C	C	A	G	T	A	C	A	G	C	C	G
Aquilaria hirta	T	T	C	C	C	T	–	G	C	C	G	T	T	C	A	C	C	G	C	C	C	G	G	T	C	T	G	A	C	C	G
Aquilaria malaccensis	T	C	C	C	C	C	T	G	C	C	C	C	T	T	A	C	T	G	C	T	C	G	G	T	A	T	G	A	C	T	G
Aquilaria microcarpa	T	C	C	C	C	T	G	A	T	T	G	T	T	T	A	C	C	G	C	C	C	G	G	T	A	T	G	A	T	C	G
Aquilaria subintegra	T	T	T	T	C	T	T	G	T	C	G	T	C	C	C	C	C	C	G	C	C	A	G	T	A	C	A	G	C	C	G

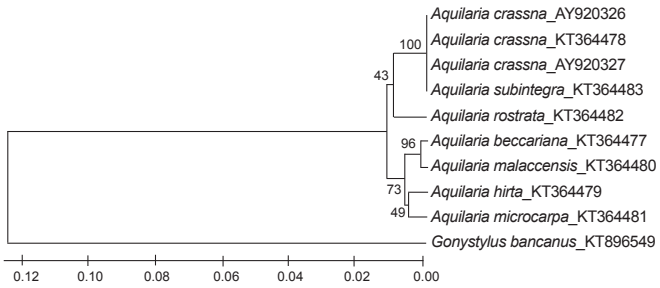

Fig. 1 UPGMA tree constructed using the nuclear ribosomal ITS sequences of ten *Aquilaria* accessions. *Gonystylus bancanus* (KT896550) included as outgroup. Bootstrap values (1 000 replicates) are shown at the branches. GenBank accession numbers are indicated at the end of each species name.

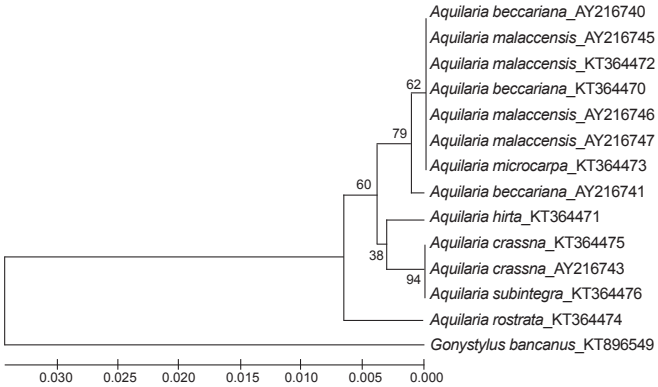

Fig. 2 UPGMA tree constructed using the intergenic spacer region *trn*L-*trn*F sequences of 14 *Aquilaria* accessions. *Gonystylus bancanus* (KT896550) included as outgroup. Bootstrap values (1 000 replicates) are shown at the branches. GenBank accession numbers are indicated at the end of each species name.

TAXONOMIC TREATMENT

Modified from *Aquilaria rostrata* Ridl., Fl. Malay. Penin. 3 (1924) 148.

Type. *Ridley 16264* (K, K00080224; SING, SING0054602), Pahang, Wray's Camp, Gunung Tahan, July 1911.

Tree 2–5 m high, 3–4 cm diam. Branchlets pubescent, glabrescent. *Leaves* alternate, subcoriaceous, glabrous, rather shining on both surface, elliptic or lanceolate, rarely ovate-oblong, 6.5–14 cm wide by 2.5–5 cm long (Fig. 3a); base obtuse, cuneate to attenuate; apex acuminate, the acumen up to 1.5 cm long; secondary nerves 16–many pairs, simple or rarely branched, spreading or slightly curved and ascending, elevated beneath and visible above; veins visible beneath and obscure above; petiole 3.5–7 mm. *Inflorescence* axillary. Flower yellowish white. *Pedicels* c. 3–5 mm, brownish hairy. *Floral tube* cylindric, 5–6 mm long, splitting on one side, glabrous outside sparsely puberulous inside (Fig. 3b). Calyx lobes slightly oblong or oblong, c. 1.5 mm long (Fig. 3c, d), puberulous on both surfaces. *Petaloid appendages* unknown. *Stamens* sessile. *Fruits* (young) obovate, obovate-oblong or oblanceolate, including the stipe 3 by 0.75–1.5 cm, greenish outside when fresh, puberulous, long-narrowed towards the base, apex beaked, acuminate or caudate (Fig. 3c, d). *Seeds* slightly ellipsoid or ellipsoid-oblong, 9–10 by 4–7 mm (excluding the appendage), brownish (Fig. 3e, f), puberulous, acuminate, base attenuate and elongate into a slender appendage, glabrous.

Distribution — Peninsular Malaysia: Pahang, Wray's Camp, Gunung Tahan; Terengganu, Besut, Gunung Tebu.

Vernacular names — Peninsular Malaysia: Karas gunung, Chandan gunung.

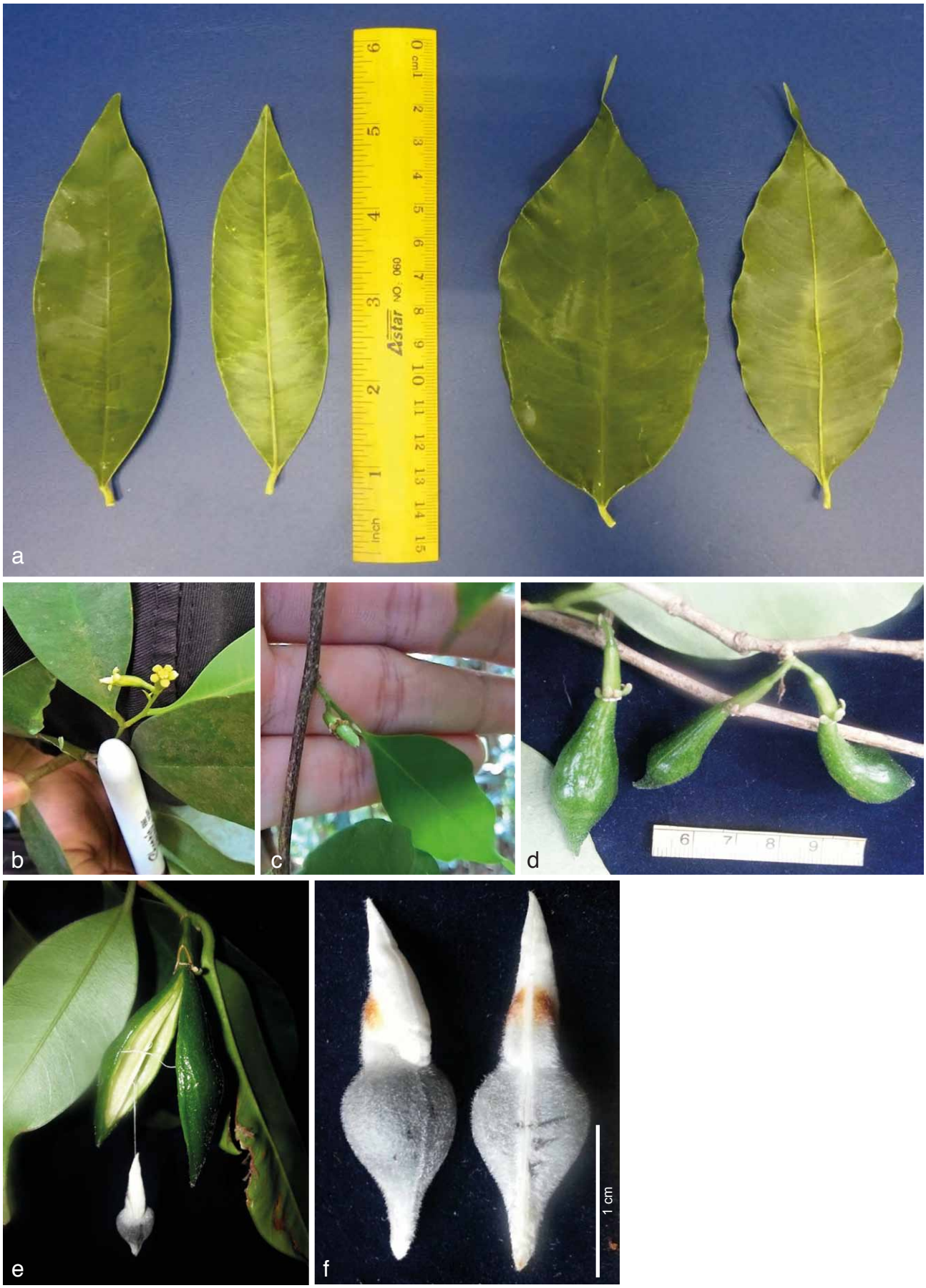

Fig. 3 Morphology of leaf and reproductive parts of *Aquilaria rostrata* population discovered in Terengganu. a. Leaf shape and size of two groups of individuals; b. opening flower; c. emerging young fruit; d. capsules with beak-like apex; e. capsule with acuminate apex and dangling seed; f. seed covered with fine white hair. — Photos d–f: Salleh Endot.

Table 3 Nucleotide variation in the intergenic spacer *trn*L-*trn*F region of *Aquilaria rostrata* compared to six different *Aquilaria* species. Species-specific mutations for *A. rostrata* are indicated in dark grey, while mutations shared with other *Aquilaria* species are indicated in light grey. GenBank accession numbers are as listed in Table 1.

Nucleotide positions	38	62	193	335	340	375	379	421	426	434
Aquilaria rostrata	G	G	A	T	G	C	T	A	G	G
Aquilaria beccariana	T	G	C	T	T	A	G	A	T	A
Aquilaria crassna	T	G	A	T	G	A	T	C	T	A
Aquilaria hirta	T	G	A	G	T	A	T	A	T	A
Aquilaria malaccensis	T	G	C	T	T	A	G	A	T	A
Aquilaria microcarpa	T	G	C	T	T	A	G	A	T	A
Aquilaria subintegra	T	G	A	T	G	A	T	A	T	A

Ecology — Natural habitat at high elevation (> 700 m asl).
Phenology — Flowering in May; fruiting in June–July.

Additional specimens examined. Malaysia, Terengganu, Besut, Gunung Tebu, *Lee SY*, *Mohamed R & Salleh E*, *FBL03001–FBL03003* (FBL, SING), *FBL03004–FBL03005* (FBL), 28 Apr. 2015; Terengganu, Besut, Gunung Tebu, *Mohd Nasir M*, *Salleh E & Mat Yusop Y*, *SE2072* (FBL, KEP, SING), 8 June 2015.

Conservation status — *Aquilaria rostrata* was only known from a single collection made by H.N. Ridley in July 1911. This collection was made from a location in the Taman Negara National Park, in Pahang, Peninsular Malaysia. No collection was recorded since then and consequently it has been suggested to be extinct in the wild due to the heavy exploitation of agarwood resources (Lim 2012).

The rediscovered *A. rostrata* in this study was from a sustainably managed logged-over forest in Besut Province, Terengganu. The population was little disturbed by agarwood collectors until recently when logging trails to the nearby forest compartment created easy access. Although no trees were found producing agarwood, we observed signs of pathological infection in trees of just 3 m tall. Several trees had fresh shallow wounds, suggesting agarwood collectors had just started targeting this population. During the first collecting visit, a rough count was made of about 50 individual trees within this population, including juvenile and mature trees. These trees were just standing about 2–5 m tall with dbh about 3–4 cm. Larger trees were not found. Given that the area has been a logged-over forest, we have no information about the presence of any trees above ten metres tall in the past. Ridley (1924) did not mention the size of the *A. rostrata* tree that he collected. However, during the second collection trip, almost all trees in the population were fruiting, suggesting that they are mature trees and that the species does not grow large. In addition, our observation suggests that mass-flowering phenomenon occurs in *A. rostrata* similar to *A. malaccensis* (Lau 2015). A brief survey conducted during the second collection revealed that the population is confined to areas at 700–750 m asl, thus efforts to conserve this species have to consider preserving the habitat of this elevation-specific species.

Note on morphology — The Terengganu population varies in leaf and fruit characteristics. Some individuals have broad elliptic leaves, and oblanceolate and apex acuminate fruits (Fig. 3a, e), while others have lanceolate leaves, and obovate and beak-like fruits (Fig. 3a, d). The latter form resembles the type collection of *A. rostrata* from Pahang, where the fruit had a beak-like apex, also described as club-shaped. Unfortunately, Ridley's type specimen is in too poor condition to verify its floral characteristics. Other morphological differences observed between the type specimen and the specimens collected from the newly discovered population are summarized in Table 5.

Table 4 Maximum identity match obtained when comparing the ITS and *trn*L-*trn*F sequences of *Aquilaria rostrata* from this study to other *Aquilaria* species from NCBI GenBank.

DNA region	Identity match (%)	Species	GenBank accession number
ITS	98	*Aquilaria malaccensis*	KM887433
	95	*Aquilaria crassna*	AY920326
		Aquilaria rugosa	AY920328
		Aquilaria sinensis	EF645836
		Aquilaria yunnanensis	EF645834
*trn*L-*trn*F	99	*Aquilaria crassna*	AY216743
	98	*Aquilaria beccariana*	AY216740
		Aquilaria citrinicarpa	AY216742
		Aquilaria malaccensis	AY216747
		Aquilaria parvifolia	AY216748
		Aquilaria sinensis	EU6526677
		Aquilaria urdanetensis	AY216750
	97	*Aquilaria khasiana*	AY216762
		Aquilaria yunnanensis	EU652881

Table 5 Morphological differences between *Aquilaria rostrata* from Pahang and Terengganu.

	Pahang (described by Ridley 1924)	Terengganu (this study)
Leaves	Lanceolate, rarely ovate-oblong, 6.5–10 by 2.5–10 cm	Elliptic or lanceolate, 10.5–14 by 3.5–4.5 cm or 10–12 by 4.5–5 cm
Pedicels	c. 3 mm long	4–5 mm long
Calyx lobes	Slightly oblong	Oblong
Floral tube	6 mm long	5 mm long
Fruit	Obovate-oblong or oblanceolate, including stipe 3 by 0.75–1.5 cm, brownish hairy outside, long-narrowed towards the base, apex beaked	Oblanceolate or obovate, including stipe 3.2 by 1.2 cm, greenish hairy outside, apex acuminate or caudate, beak-like
Seed	Slightly ellipsoid-oblong, 10 by 4 mm	Ellipsoid, 9 by 7 mm

KEY TO THE AQUILARIA SPECIES IN PENINSULAR MALAYSIA

1. Small tree 2–10 m tall, to 15 cm diam 2
1. Medium to large tree up to 40 m tall, 30–60 cm diam . . . 3
2. Leaves elliptic-oblong, ovate-oblong, dull and pubescent beneath especially on the midrib, nerves and veins, sometimes glabrescent, shining on the upper surface, base cuneate to obtuse or rounded; apex acuminate. Fruit protruding from the floral tube, oblanceolate, base attenuate, apex abruptly acute . . . . . *A. hirta*
2. Leaves lanceolate, ovate-oblong, glabrous, shining on both surfaces, base obtuse, cuneate to attenuate; apex acuminate. Fruit protruding from the lateral slit of floral tube, obovate, obovate-oblong or oblanceolate, base long-narrowed, apex beak-like . . . . . *A. rostrata*
3. Floral tube (calyx tube) cylindric. Calyx lobes much shorter than the tube, spreading. Leaves papery, usually glabrous on both surfaces, oblong, oblong-lanceolate or elliptic-oblong, 11–27 by 6–8.5 cm. Fruit obovoid, laterally compressed, slightly contracted in the middle, base abruptly narrowed to the elongate stipe . . . . . *A. beccariana*
3. Floral tube (calyx tube) cup- or bell-shaped. Calyx lobes slightly shorter or longer than the tube, spreading or reflexed. Leaves chartaceous, subcoriaceous, shining and glabrous above, glabrous or slightly hairy beneath, elliptic-oblong to obovate-oblong or oblanceolate, 4.5–12 by 1.5–5.5 cm. Fruit obovoid or obovoid-oblong or subcordate, never or slightly compressed, base cuneate 4
4. Fruit obovoid or obovoid-oblong, never compressed, 3–4 by 2–3 cm. Stamens longer than the petaloid appendages with anthers emerging from the throat of the floral tube . . . . . *A. malaccensis*
4. Fruit subcordiform, slightly compressed, 0.8–1.5 by 1–1.5 cm. Stamens shorter than or as long as petaloid appendages with anthers included in the floral tube . . . . *A. microcarpa*

CONSERVATION STATUS OF AQUILARIA IN PENINSULAR MALAYSIA

Malaysia is rich in *Aquilaria* diversity (Table 6) and this leads to many names given by the local people. The general local name in the Peninsular Malaysia for *A. malaccensis* is 'karas' or 'depu'. 'Karas bulu' or 'chandan bulu' is dedicated to *A. hirta* and 'karas/chandan gunung' is assigned to *Aquilaria* trees found at high elevations such as natural forests in the mountainous region. The precious agarwood produced from *Aquilaria* tree is known as 'gaharu'. In 2005, the Forest Department of Peninsular Malaysia (FDPM) reported that the number of wild *Aquilaria* trees in natural forests of Peninsular Malaysia stood at more than 3 million individuals. This was based on a nation-wide inventory conducted between 2002 and 2004 (FDPM 2005). By now, a decade later, this number is expected to have steeply declined due to illegal agarwood harvesting activities and diminishing natural habitats due to conversion of forest to agriculture land.

Table 6 *Aquilaria* conservation status in Peninsular Malaysia and the IUCN Red list of Threatened Species™.

Species	Peninsular Malaysia[1]	IUCN[2]
A. beccariana	Data deficient	Vulnerable (A1d)
A. hirta	Vulnerable (A4cd)	Vulnerable (A1d)
A. malaccensis	Vulnerable (A4cd)	Vulnerable (A1cd)
A. microcarpa	Data deficient	Vulnerable (A1d)
A. rostrata	Data deficient	Critically endangered (B1ab(v))

[1] *Aquilaria* conservation status in Peninsular Malaysia (Lau & Chua 2011).
[2] IUCN conservation status for *Aquilaria* based on IUCN (2015).

It is more difficult to determine the conservation status for *A. beccariana*, *A. microcarpa* and *A. rostrata* as the information is limited. Although the two former species are recorded as abundant in Sabah and Sarawak, there is little available information on their presence in Peninsular Malaysia. Similar to *A. rostrata*, *A. microcarpa* in Peninsular Malaysia is also known from a single herbarium specimen, collected from Mersing, Johor. Another species reported from Mersing is *A. beccariana* (Faridah-Hanum et al. 2009). However, our excursions conducted in March 2015 revealed the reported site in present day as located next to an agriculture plantation. This could explain the disappearance of these two species from Mersing, or else they could not be found due to inaccessibility of their existing populations. Previous assessments by the forest department also gathered no new information on natural *Aquilaria* trees within that area. *Aquilaria beccariana* was also reportedly found in Kota Tinggi, Johor, but new collections or updates are not available to ascertain its status.

For *A. rostrata*, the forest department had carried out an extensive sampling effort but failed to relocate this species at Wray's Camp, Gunung Tahan, Pahang. The population is believed to have disappeared owing to illegal agarwood harvesting (M. Mohd Nasir, FDPM, pers. comm.). Due to the difficulty of obtaining information on *Aquilaria* natural stands in Peninsular Malaysia, *A. hirta* and *A. malaccensis* have been considered for the conservation status Vulnerable (VU), while the remaining three species have been given the temporary status Data Deficient (DD) (Lau & Chua 2011). However, the IUCN Red List of Threatened Species (IUCN 2015) categorizes all the five species as Vulnerable (VU), except for *A. rostrata*, which is classified as Critically Endangered (CR) (Table 6).

CONCLUSION

Aquilaria rostrata appears to be a highly confined species within its natural habitat due to its preference to high elevation. This perhaps safeguards the species from exploitation by human activities, which has posed serious threats to its related species in the lowland. Ironically, it was the destructing activities of the natural habitat through logging that provided us with access to this unknown population. Other populations may exist unobserved given the difficulty of access to its natural habitat. We report the first encounter with a surviving *A. rostrata* population, since its initial recognition in 1924. This finding brings comfort and new hope, as we know now that the future is not lost for our natural heritage, *A. rostrata*.

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