A taxonomic study of Quercus (Fagaceae) in Vietnam based on molecular phylogeny and morphological observations

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Ph.D. Thesis

A taxonomic study of *Quercus* (Fagaceae) in Vietnam based on molecular phylogeny and morphological observations

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Preface

The genus *Quercus*, with more than 500 species, is one of the largest genera in the family Fagaceae. The species is widely distributed in the world and often dominant in temperate deciduous forests in eastern North America, Europe and Asia, Mediterranean, desert scrub in Europe, Mexico and adjacent regions, and tropical montane forests in Southeast Asia. Species delimitation of *Quercus* has been based on morphological characters and some genetic markers.

In Vietnam, botanical surveys had a long blank period from 1930s to 2000s when 40 species of *Quercus* were reported. The taxonomic treatment of the genus *Quercus* in Vietnam remains to be revised, because of insufficiently available materials and wide morphological variation in leaves and fruits, which led to confusions of taxonomy and difficulties in identification and numerous scientific names are still controversial.

In this thesis, I revised species taxonomy of the genus *Quercus* in Vietnam using both morphological comparison and molecular phylogenetic analysis. This study was based on observations on recent collections obtained from a series of field surveys in Vietnam and surrounding countries, literature review and examination of type specimens of each species in the herbaria as well as digital specimen images on JSTOR Global Plants. Both classic barcoding regions (*rbc*L, *mat*K and ITS) and genome-wide markers obtained using the next generation sequencing platform (MIG-seq) were used to clarify the relationships among the closely related species of *Quercus* in Vietnam.

This thesis consists of four chapters. In Chapter 1, I describe morphological and molecular evidence for an unknown species collected from Gibbon Area, Trung Khanh District, Cao Bang Province, north-eastern Vietnam, which was not assignable to any of the previously known taxa in Vietnam and its surrounding countries. Based on this evidence, a new species is described as *Quercus trungkhanhensis* Binh & Ngoc with photographs, conservation status, and the DNA barcode of *mat*K and ITS.

In Chapter 2, I revise the taxonomy of the *Q. langbianensis* complex based on evidence obtained from field observations, morphological studies and molecular data from both classic and next-generation DNA markers. In conclusion, we distinguished 10 species in the *Q. langbianensis* complex, including the seven species previously treated as synonyms of *Q. langbianensis* (Plant List 2013) and the remaining three undescribed species that are described as *Q. baolamensis* Binh

& Ngoc, *Q. bidoupensis* Binh & Ngoc and *Q. honbaensis* Binh, Tagane & Yahara. Also, a key for each species in the complex was provided and six species of the complex were lectotypified.

In Chapter 3, a new species is reported from Xuan Lien Nature Reserve and two species are newly recorded from Ba Vi National Park. A new species is described as *Quercus xuanlienensis* Binh, Ngoc & Bon. The two newly recorded species to the country are *Q. disciformis* Chun & Tsiang. and *Q. bella* Chun & Tsiang. In addition to the morphological examination, genome-wide markers (MIG-seq) of the three species were compared with those of 20 species in Vietnam to confirm that the three species are divergent and thus distinct from the other species.

In Chapter 4, I revise the taxonomy of the genus *Quercus* in Vietnam based on both molecular and morphological evidence, as well as our field observations. Forty-three *Quercus* species were enumerated in Vietnam, among which ten species were undescribed and tentatively named as *Q. pseudocamusiae*, *Q. fansipanensis*, *Q. haivanensis*, *Q. ngoclinhensis*, *Q. semiundulata*, *Q. sontraensis*, *Q. theifolia*, *Q. tiepii*, *Q. verticillata*, and *Q. vuquangensis*.

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Abstract

The genus *Quercus*, with more than 500 species, is one of the largest genera in the family Fagaceae. The species is widely distributed in the world and often dominant in temperate deciduous forests in eastern North America, Europe and Asia, Mediterranean and desert scrub forest in Europe, Mexico and adjacent regions, and tropical montane forests in Southeast Asia. Species delimitation in *Quercus* has been based on morphological characters and some genetic markers. However, those characters and markers often exhibit broad ranges of intraspecific variation most likely due to co-ocurrance of some species in ecologically heterogeneous habitats and their interspecific hybridizations in many species pairs. As for genetic markers, widely used DNA barcoding regions such *rbc*L*, mat*K and ITS do not always provide clear discriminating signals at the species level in *Quercus* because of the existence of paralogous loci.

In Vietnam, botanical surveys had a long blank period from 1930s to 2000s when 40 species of *Quercus* were reported. The taxonomic treatment of the genus *Quercus* in Vietnam remains to be revised, because of insufficiently available materials and wide morphological variation in leaves and fruits, which led to confusions of taxonomy and difficulties in identification and numerous scientific names are still controversial.

In this thesis, species taxonomy of the genus *Quercus* in Vietnam is revised based on the morphological comparison and molecular phylogenetic analysis. The species identification was consulted based on our recent collections obtained from a series of our field surveys in Vietnam and surrounding countries, literature review and type specimens of each species in the herbaria as well as digital specimen images on JSTOR Global Plants. Both three classic barcoding regions and genome-wide markers using the next generation sequencing platform (MIG-seq) were used to clarify the relationships among the closely related species of *Quercus* in Vietnam.

Based on morphological and molecular evidence, the taxonomy of *Quercus langbianensis* complex and its relatives or similarities in Vietnam and Cambodia was revised and a key for each species in the complex was provided. We also identified 43 species of *Quercus* from Vietnam, including 15 undescribed species newly described as *Q. baolamensis*, *Q. bidoupensis*, *Q. honbaensis*, *Q. trungkhanhensis*, and *Q. xuanlienensis,* and tentatively named as *Q. pseudocamusiae*, *Q. fansipanensis*, *Q. haivanensis*, *Q. ngoclinhensis*, *Q. semiundulata*, *Q. sontraensis*, *Q. theifolia*, *Q. tiepii*, *Q. verticillata*, and *Q. vuquangensis*. Two species *Q. bella* and *Q. disciformis* are newly recorded from Vietnam. In addition, the following six species that are not

listed in the Illustrated Flora of Vietnam were confirmed as distinct: *Q. baniensis*, *Q. blaoensis*, *Q. dilacerata*, *Q. donnaiensis*, *Q. platycalyx*, *Q. sessilifolia* and *Q. xanthoclada*.

According to the evidence provided, the species of *Quercus* in Vietnam rose from 40 to 59 species. This figure shows that Vietnam is the center of species diversity of *Quercus* in Asia next to China.

Keywords: DNA barcoding, Fagaceae, MIG-seq, *Quercus,* new record, new species, taxonomy, Vietnam.

Chapter 1. *Quercus trungkhanhensis* (Fagaceae), a new species from Cao Vit Gibbon Conservation Area, Cao Bang Province, north-eastern Vietnam.

Abstract

A new species, *Quercus trungkhanhensis* Binh & Ngoc (Fagaceae), from Cao Vit Gibbon Conservation Area, northeastern Vietnam is described. It is morphologically similar to *Q. engleriana* Seemen and *Q. marlipoensis* Hu & Cheng in having scaly cupules and in the shape, texture and glabrescence of the leaves, but distinguished from the former particularly by the size and morphology of the fruits (acorns and cupules) and the latter by smaller leaves with fewer lateral veins. *Quercus trungkhanhensis* is also similar to *Q. franchetii* Skan in fruit morphology, but differs in having glabrescent leaves.

Keywords

Cao Vit Gibbon Conservation Area, Fagaceae, new species, *Quercus*, Vietnam.

Introduction

Quercus Linnaeus (1753) (Fagaceae), with 400-500 species (Nixon 1993, Valencia-A et al. 2016), is one of the largest genera in the Fagaceae. The genus is characterized by pendulous staminate inflorescences, carpellate flowers always solitary, capitate or dilated stigma and indehiscent cupules (Huang et al. 1999, Phengklai 2008). Some species are often dominant in various forest types, including temperate deciduous forests in eastern North America, Europe and Asia, Mediterranean and desert scrub forest in Europe, Mexico and adjacent regions, and tropical montane forests in Southeast Asia (Nixon 1993, Hubert et al. 2014, Valencia-A et al. 2016,). Besides their economic and ecological importance, species of *Quercus* are also considered in many countries as cultural and patrimonial resources (Hubert et al. 2014).

Vietnam is known for the high diversity of species of Fagaceae; 216 species in 6 genera, including 44 species of *Quercus* have been recorded (Ho 2003, Ban 2005, Li and Coombes 2016, Ngoc et al. 2016). Recently, taxonomic studies of Fagaceae in Vietnam have been undertaken by

Deng et al. (2010), Linh et al. (2013), Vuong and Xia (2014), Li and Coombes (2016) and Ngoc et al. (2016), but the taxonomy of *Quercus* remains to be revised.

Here, we report a new species of *Quercus* from Cao Vit Gibbon Conservation Area, located at Trung Khanh District, Cao Bang Province, northeastern Vietnam (Fig. 1.1). The conservation area was established in 2007 to strengthen conservation for the Cao Vit gibbon (*Nomascus nasutus*) and covers 7,600 ha of limestone ground where five types of vegetation are found (Tu et al. 2009): subtropical evergreen broad leaved forests in valleys, subtropical bamboo forests in valleys, limestone subtropical evergreen mixed forests, tropical evergreen shrub savannahs and tropical secondary evergreen grasslands. Within the conservation area, 960 species of vascular plants, of which 34 species are listed as threatened in Vietnam's Red Data Book (Ban et al. 2007, Tu et al. 2009), belonging to 144 families have been recorded.

During a botanical survey at Cao Vit Gibbon Conservation Area in 2016, we discovered an undescribed species of *Quercus*, based on morphological comparison with morphologically similar species, which we describe and illustrate below as *Quercus trungkhanhensis* Binh & Ngoc. We also provide DNA sequences of the two regions of ITS and *mat*K for DNA barcoding, and assess its conservation status using IUCN Red List criteria (IUCN 2001).

Material and methods

Morphological observations

To verify the validity of the new species, we thoroughly reviewed the literature (Camus 1936-1954, Soepadmo 1972, Ho 2003, Huang et al. 1999, Ban 2005, Phengklai 2008) related to *Quercus* in Vietnam and surrounding countries. Based on the cupule morphology, *Quercus trungkhanhensis* is considered to be a member of subgenus *Quercus* (scale-cup oaks; Nixon 1993). According to the key in the Flora of China (Huang et al. 1999), *Q. trungkhanhensis* corresponds to *Q. engleriana* Seemen or *Q. marlipoensis* Hu & Cheng in the persistent leathery leaves with acute apex, cupule bracts scale-like, petiole 1–3 cm long, mature leaf blade abaxially glabrous or early glabrescent. According the acorn-based key in the Flora of Thailand (Phengklai 2008), *Q. trungkhanhensis* corresponds to *Q. franchetii* Skan in scaly cupule wall, cup-shaped cupule, each infructescence with (1 or)2 acorns, and serrate leaf margin. We therefore compared *Q.*

trungkhanhensis with *Q. engleriana*, *Q. marlipoensis* and *Q. franchetii.* For morphological comparison, we examined specimens using the websites of JSTOR Global Plants (http://plants.jstor.org) and the Chinese Virtual Herbarium (hereafter CVH: http://www.cvh.org.cn/). We also examined more than two hundred dried specimens kept in the herbaria DLU, FU, HN, P and VNM.

DNA barcoding

For DNA isolation, a piece of leaf was collected and desiccated using silica gel in the field. DNA was isolated by the CTAB method (Doyle and Doyle 1987) with minor modifications described in Toyama et al. (2015). We determined sequences of two DNA barcode regions; the internal transcribed spacer (ITS) and the large subunit of maturase K (*mat*K) using the published protocols of Rohwer et al. (2009) and Dunning and Savolainen (2010) with a minor modification using Tks GflexTM DNA Polymerase (TAKARA, Japan) in the PCR amplification.

Phylogenetic analysis

We constructed a phylogenetic tree using nucleotide sequences of the DNA barcoding regions of ITS (487 bp) for 9 species including 8 species of *Quercus* and one species of *Lithocarpus* (Table 1.2). In addition to *Quercus trungkhanhensis*, we included *Q. engleriana*, *Q. franchetii* and three other species of subgenus *Quercus* for which ITS sequences were available in GenBank. No ITS sequence of *Q. marlipoensis* was available in GenBank. Two species of *Quercus* subgenus *Cyclobalanopsis* and *Lithocarpus dahuoaiensis* Ngoc & L. V. Dung were used as outgroups. Sequence alignment was performed by ClustalW with default parameter implemented in MEGA v 7.0 (Kumar et al. 2016).

The Neighbor-joining method (Saitou and Nei 1987) with Maximum Composite Likelihood distance matrix (Tamura et al. 2004) implemented in MEGA v 7.0 was used to construct the phylogenic tree. Confidence values for individual branches were determined by bootstrap analysis with 10,000 times resampling of the data.

Results

The comparison of characters among *Q. trungkhanhensis, Q. engleriana* and *Q. franchetii* is shown in Table 1.1. Among the four syntypes of *Q. engleriana* available on the webpage of JSTOR Global Plants (A. *Henry 5682*, 1885-1888, China, BM, US and two specimens at GH), *Henry 5682A* (GH) is the only fruiting specimen. *Quercus trungkhanhensis* is distinct from *Henry 5682A* in the size and morphology of the mature fruits. The mature fruits of *Henry 5682A* (*Q. engleriana*) are 12.5–13 mm long, with cupules 3.8–5.5 mm tall and 8.5–10 mm wide, acorns 5–7 mm long above the cupule and 6.5–7.5 mm wide, and the stylopodium is ca. 2 mm long. In *Q. trungkhanhensis* the mature fruits are 16–22 mm long, with cupules 8–10 mm tall and 12–14 mm wide, acorns 8–12 mm long above the cupule and 10–12 mm wide, and the stylopodium is ca. 2 mm long. The acorns are ovoid and acute at apex in *Q. engleriana*, but cylindrical and slightly concave at apex in *Q. trungkhanhensis.* The cupules are cup-shaped and relatively loosely covered with scales on the lower half in *Q. engleriana* but more cylindrical and tightly covered with scales in *Q. trungkhanhensis* (Fig. 1.2D–F). There are many images of *Q. engleriana* in CVH in showing the above distinctions to be mostly stable. As for fruit morphology of *Q. engleriana*, *PE 00297544* (Nanchuan, Chongqing, alt. 1750 m, 6 Oct. 1957, J.-H. Xiong & Z.-L. Zhou *93826* (PE): http://www.cvh.org.cn/spm/PE/00297544) has exceptionally more cylindrical acorns but the acorns are less than 10 mm long, as in typical *Q. engleriana*. In *Henry 5682A*, the fruiting branchlets have 12 terminal or lateral buds that are narrowly ovoid, 5–9 mm long and 3–6 mm wide, whereas in *Q. trungkhanhensis* the buds are broadly ovoid, 3–4 mm long and 2–3 mm wide (Fig. 1.2A, Fig. 1.3B). The shape of the narrowly ovoid buds is stable among specimens of *Q. engleriana* in CVH. The young branchlets of *Q. trungkhanhensis* are appressed hairy with yellowish brown hairs (Fig. 1.2A), but the branchlets of *Q. engleriana* are yellowish gray tomentose. *Quercus trungkhanhensis* is also distinct from *Q. engleriana* in having nearly glabrous leaves on fruiting branchlets; *Q. engleriana* usually retains dense hairs along the abaxial veins and on the petioles. According to the images on the CVH website, hairiness of the leaves of fruiting branchlets is somewhat variable, but *Q. engleriana* retains dense hairs at least in the vein axils.

The digital images of the holotype (*PE00039496*) of *Quercus marlipoensis* available on the CVH webpage shows much larger leaves with more lateral veins (leaf blade 12–22 × 6–11 cm, with 11–16 pairs of lateral veins) than *Q. trungkhanhensis* (leaf blade 9–15 × 2–5 cm, with 8–10 pairs of lateral veins) and *Q. engleriana.* Among four specimens of *Quercus marlipoensis* at PE,

PE 00022946 (http://www.cvh.org.cn/spm/PE/00022946) has three cupules which are 13–15 mm wide and larger than those of *Q. engleriana* (8.5–10 mm wide) and as large as *Q. trungkhanhensis* (12–14 mm wide). In *PE 00022946* the buds are narrowly ovoid (12 × 7 mm) as in *Q. engleriana*. In all four specimens, the leaves and petioles are almost glabrous as in *Q. trungkhanhensis* and the branchlets are less hairy than in *Q. trungkhanhensis* and *Q. engleriana.*

Six images of syntypes of *Quercus franchetii* are on the webpage of JSTOR Global Plants (*Henry A 9298*, Yunnan, China, A(2), K(2), NY, US). *Quercus trungkhanhensis* is easily distinguished from *Q. franchetii* by having nearly glabrous leaves on the fruiting branchlets (vs. densely yellowish gray tomentose on the lower surface). Among the six syntypes, two specimens (A and K) have mature fruits have cup-shaped, somewhat cylindrical cupules 8–9 mm and tightly covered with scales and cylindrical acorns 10–12 mm long and 8–9 mm wide and slightly concave at the apex.

The Neighbor-joining tree base on ITS (Fig. 1.4) showed that *Quercus trungkhanhensis* is sister to *Q. franchetii* with 67% bootstrap probability and *Q. engleriana* is sister to those two species with 77% bootstrap probability. *Quercus trungkhanhensis* differed from *Q. franchetii* in four nucleotides and from *Q. engleriana* in ten nucleotides.

Taxonomy

Quercus trungkhanhensis Binh & Ngoc, **sp. nov.** —Figs. 1.2 & Fig. 1.3.

Diagnosis. Quercus trungkhanhensis is morphologically similar to *Q. engleriana* and *Q. marlipoensis*, both distributed in China, in having persistent leaves, acuminate leaf apex, cupules covered with scales, petiole 1–3 cm long, mature leaf blades abaxially glabrous or early glabrescent, and leaf blade leathery; *Q. trungkhanhensis* differs from *Q. marlipoensis* in having smaller leaves, and differs from *Q. engleriana* in having larger, cylindrical slightly concave at apex (vs. ovoid and acute at the apex), larger cupules tightly covered with scales (vs. relatively sparsely covered with scales on basal half), broadly ovoid buds (vs. narrowly ovoid), and yellowish brown appressed hairy branchlets when young (vs. yellowish gray tomentose).

Type. VIETNAM. Cao Bang Province, Trung Khanh District, limestone subtropical evergreen mixed forests of Cao Vit Gibbon Conservation Area, 22°54'55"N, 106°31'28"E, alt. 767 m, 6 Nov. 2016, *Binh HT, Ngoc NV, Tai VA, Son HT V6066* (holotype KYO!, isotypes DLU!, FU!).

Description. Tree, 5–10 m tall, 15 cm in girth. Bark pale gray, deeply longitudinally furrowed. Buds broadly ovoid, ca. 3–4 mm long, ca. 2–3 mm in diam.; scales in 4–6 rows, imbricate, ovate-triangular, ca. 3 × 2.5 mm, apex obtuse, margin ciliate, appressed hairy on both surfaces. Twigs densely yellowish brown stellate hairy when young, later glabrescent, lenticellate. Leaves alternate; petiole 1.8–2.4 cm long, densely yellowish brown hairy when young, glabrescent later; blade leathery, ovate or ovate-elliptic, (7–)9–12.5 × 2.4–5.8 cm, base rounded or shallowly cordate, margin serrate in upper (4/5–)2/3, apex acuminate, pale brown or reddish brown when dry, both surfaces glabrous except stellate hairs remaining near base of midrib on upper surface and in axils of secondary veins on lower surface; midrib on upper surface slightly raised, prominently raised on lower surface; lateral veins 8–10 pairs, prominent, at angle of 50–60(–70) degree from midrib, straight and running into marginal teeth, tertiary veins scalariform, faintly visible. Inflorescences (staminate and carpellate) not seen. Infructescences axillary or terminal, erect; peduncle woody, ca. 1 cm long; rachis 1–1.5 cm long, 4–5 mm in diam., glabrous, brownish red when fresh, blackish brown when dried, lenticellate. Mature fruits 1.6–2.2 cm long (including cupule), solitary or paired, sessile; acorns ovoid, 1–1.5 cm long, 1–1.2 cm in diam., truncate and slightly concave at apex, white tomentose when young, densely appressed hairy around stylopodium, stylopodium to 2 mm long, basal scar 5–6 mm in diam., raised; cupules cup–shaped, somewhat cylindrical, 0.8–1 cm tall, 1.2–1.4 cm in diam., enclosing 1/3 to 1/2 of the mature acorn, scales on cupule triangular, ca. 1–1.5 mm long on lower part of cupule, smaller on upper part, apex short acuminate or rarely acute, dull greenish yellow, dark purplish red near apex, densely appressed hairy with short grayish brown hairs outside, densely hairy with short yellowish brown hairs inside.

Phenology. Fruiting specimens collected in January.

Distribution and habitat. Known only from Cao Vit Gibbon Conservation Area, Trung Khanh District, Cao Bang province, Vietnam (Figure 1.1). We found only two individuals within 100 m^2 on a ridge in a limestone subtropical evergreen mixed forest, at 767 m altitude.

Etymology. The specific epithet is derived from the district name of the type locality, Trung Khanh District, Cao Bang Province, northeastern Vietnam.

GenBank accession no. Binh et al. V6066: KY867547 (ITS), LC258443 (*mat*K).

Conservation status. Critically endangered (CR). *Quercus trungkhanhensis* is known only from two individuals. The forest in the Conservation Area is currently protected under law from anthropogenic activities but the locality of *Q. trungkhanhensis* was disturbed by local people

searching for forest resources. Although additional individuals/populations of *Q. trungkhanhensis* may be discovered, it qualifies as CR under criterion B in that the area of occupancy is less than 10 km^2 at only a single location and criterion D of the population size is fewer than 50 mature individuals (IUCN 2001).

Note. Quercus trungkhanhensis is a member of *Quercus* subgenus *Quercus* (scale-cup oaks; Nixon 1993), corresponding to *Quercus* s. str., excluding *Cyclobalanopsis* (Huang et al. 1999). In Thailand (Phengklai 2008), most species of *Quercus* belong to subgenus *Cyclobalanopsis* (cycle-cup oaks; Nixon 1993). Phengklai (2008) listed only seven species of subgenus *Quercus*. Similarly, in Vietnam there are fewer species of subgenus *Quercus* than of subgenus *Cyclobalanopsis. Quercus trungkhanhensis* is easily distinguished from the eight other species of subgenus *Quercus* (*Q. acutissima* Carruth., *Q. aliena* Blume, *Q. franchetii*, *Q. kingiana* Craib, *Q. lanata* Sm., *Q. oblongata* D. Don, *Q. setulosa* Hickel & A.Camus and *Q. variabilis* Blume) in the leathery mature leaves abaxially glabrous or early glabrescent.

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Legend for tables and figures

Table 1.1. Morphological comparison between *Quercus trungkhanhensis* Binh & Ngoc, sp. nov. with *Quercus engleriana* Seemen, *Quercus franchetii* Skan and *Q. marlipoensis* Hu & Cheng. Descriptions of fruit characters are based on mature fruits.

Characters	*Q. trungkhanhensis*	*Q. engleriana*	*Q. marlipoensis*	*Q. franchetii*
Buds shape	Broadly ovoid	Narrowly ovoid[1]	Narrowly ovoid	Narrowly ovoid[4]
Twigs	Yellowish brown appressed stellate hairy	Yellowish gray tomentose[2]	Yellowish brown tomentose[2]	Yellowish gray simple and fascicled hairs[2]
Leaf margin	Serrate upper (4/5–)2/3	Serrate upper 1/2, sometime entire[2]	Scattered teeth or entire and slightly inflexed[2]	Serrate upper 1/2, setaceous at ends of teeth[2] [3]
Leaf surface	Glabrous on both surfaces except midrib and lateral veins	Densely yellowish brown pubescent[2]	Abaxially stellate tomentose along midvein[2]	Glabrous on the upper surface; densely yellowish gray tomentose below in the lower[4]
Leaf base	Rounded or shallowly cordate	Rounded, broadly cuneate, or rarely shallowly cordate[2]	Rounded[2]	Cuneate to cordate or obtuse[2] [3]
Leaf size	(7–)9–12.5 × 2.4–5.8 cm	6–16 × 2.5–5.5 cm[2]	12–22 × 6–11 cm[2]	5–12 × 2.5–6 cm[2]
Length of petioles	1.8–2.4 cm long	1–2 cm long[2]	1.5–3 cm[2]	0.2–1.4 cm long[3] [4]
Number of secondary veins	8–10 pairs	10–13 pairs[2]	16–20 pairs[2]	6–9 pairs[3] [4]

Cupule size	8–10 mm tall, 12–14 mm in diam.	3.8–5.5 mm tall, 8.5–10 mm in diam.[1]	8 mm tall, 14 mm in diam[2].	(4–)7–12 mm tall, 10–14 mm in diam.[2]
Scales of cupule	Triangular	Ovate–lanceolate[1]	Ovate[2]	Triangular[2]
Nut enclosure	Enclosing 1/3–1/2 of the nut	Enclosing 1/3–1/2 of the nut[2]	N/A	Enclosing 1/2 of the nut[2]
Apex of nut	Truncate and slightly concave	Acute[1]	N/A	Slightly concave[3] [4]
Infructescence	1–1.5 cm long, each infructescence with (1 or)2 acorns	1–5 cm long, each infructescence with 1–10 acorns[2]	N/A	1–5 cm long, each infructescence with (1 or)2 acorns[3]

[1] From the material *Henry A 5682* (GH)

[2] From the description in flora of China (*Huang* et al. 1999)

[3] From the description in flora of Thailand (Phengklai 2008)

[4] From the material *Henry A 9298* (K)

Table 1.2. List of taxa used in this study with GenBank accession number.

Subgenus	Species	GenBank accession no.
Subg. *Quercus*	*Quercus acutissima* Carruth.	AF098428
	Quercus engleriana Seemen	AY040465
	Quercus franchetii Skan	AY040464
	Quercus griffithii Hook.f. & Thomson ex Miq.	AY040490
	Quercus variabilis Blume	AY040463
Subg. *Cyclobalanopsis*	*Quercus myrsinifolia* Blume	AF098414
	Quercus lamellosa Sm.	AY040454
Outgroup	*Lithocarpus dahuoaiensis* Ngoc & L. V. Dung	KY436002

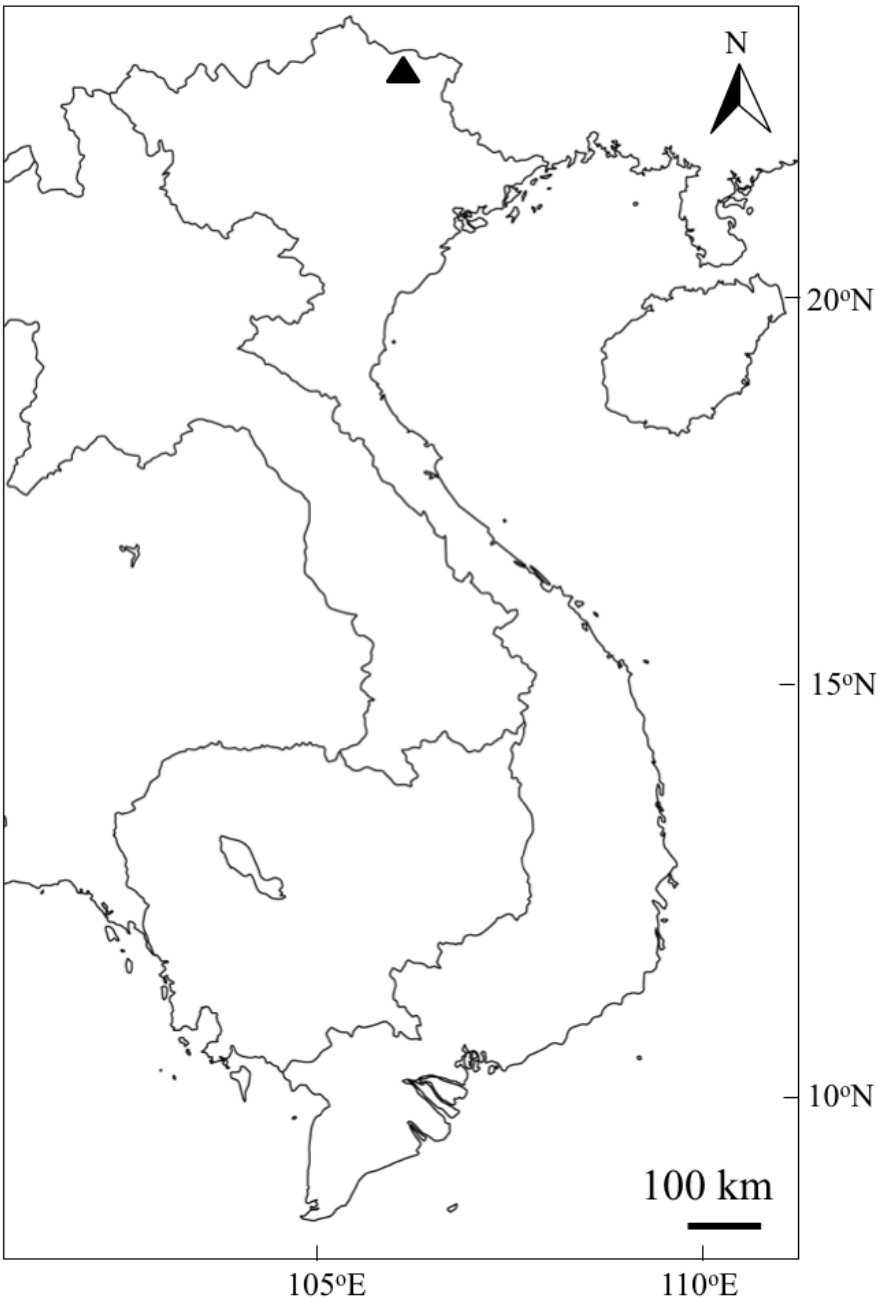

Figure 1.1. Distribution map of *Quercus trungkhanhensis* Binh & Ngoc. Black triangle: Cao Vit Gibbon Area, Trung Khanh District, Cao Bang Province.

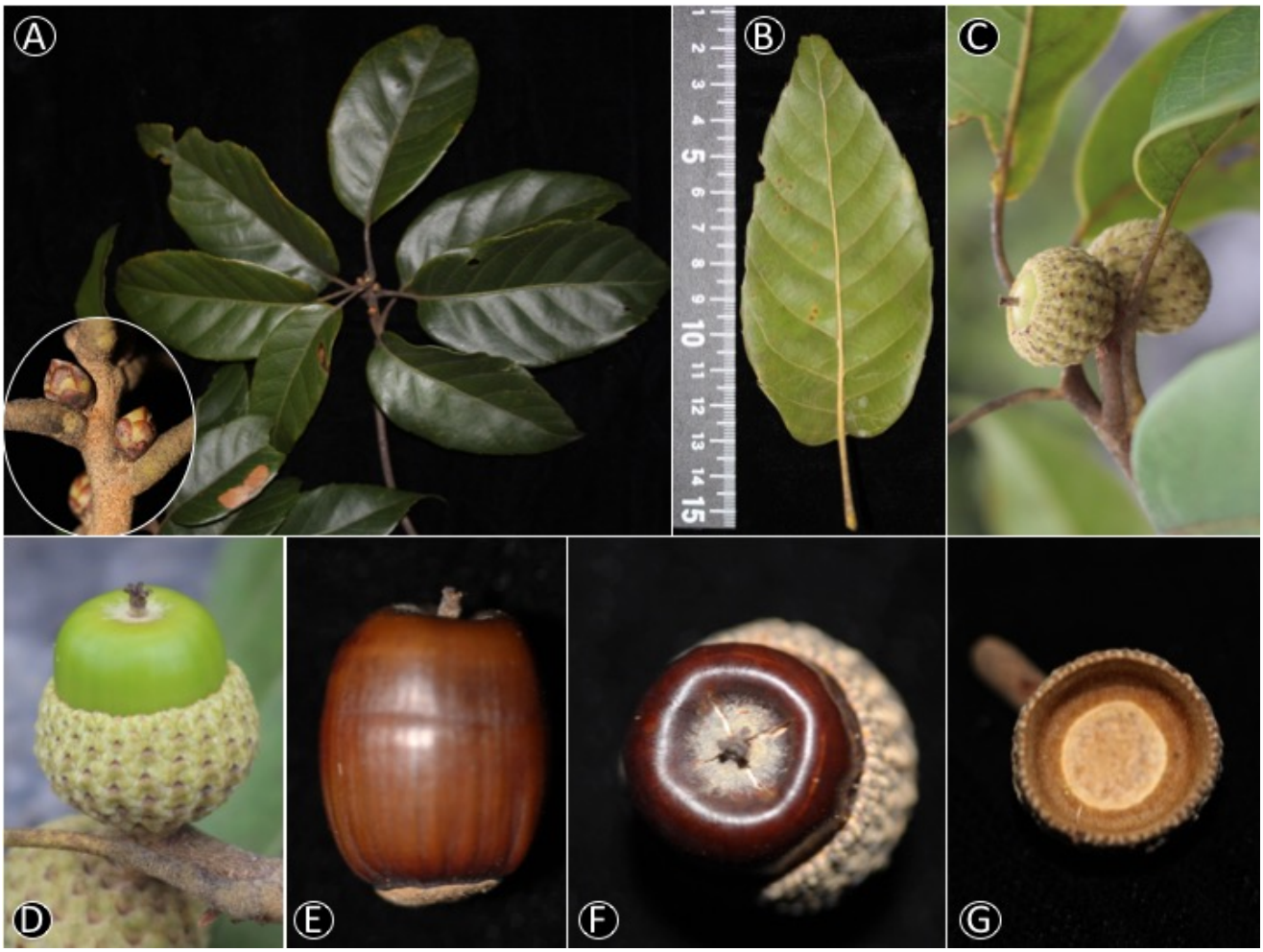

Figure 1.2. *Quercus trungkhanhensis* Binh & Ngoc (*Binh et al. V6066*). **A** Leafy twig and buds, **B** Abaxial side of mature leaf, **C** Infructescence and young fruits, **D** Mature fruit; **E** Nut (lateral view); **F** Nut (top view); **G** Inside of cupule.

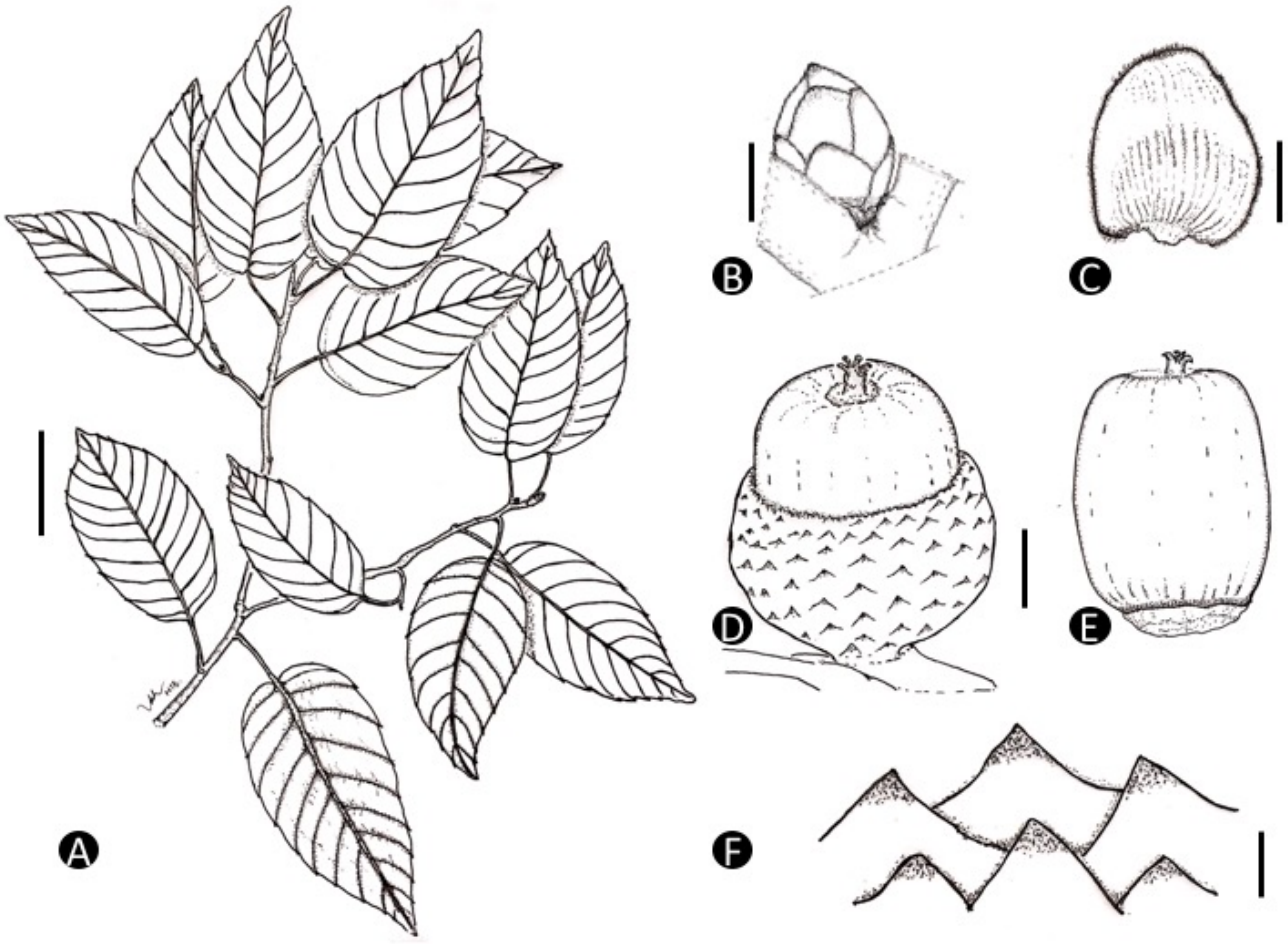

Figure 1.3. Line drawing of *Quercus trungkhanhensis* Binh & Ngoc (*Binh et al. V6066*). **A** Leafy twig, **B** Bud, **C** Bud scale, **D** Mature fruit, **E** Nut, **F** Cupule scales. Scale bars **A** = 5 cm, **B** = 2 mm, **C** = 1 mm, **D** & **E** = 5 mm, **F** = 1 mm.

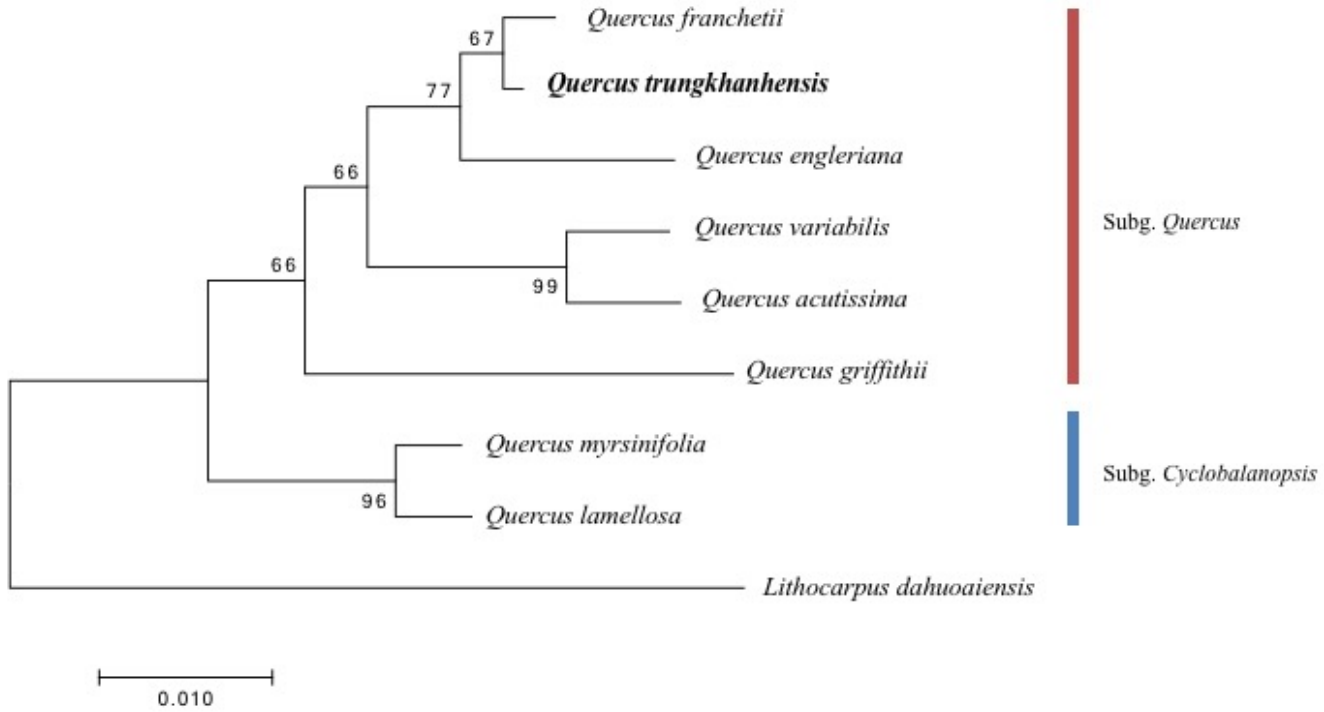

Figure 1.4. NJ tree of *Quercus trungkhanhensis* and seven species of subgenus *Quercus* and subgenus *Cyclobalanopsis* based on the data of nuclear ribosomal ITS region. Branches are labeled with bootstrap support (% of 10,000 replicates)

Chapter 2. A taxonomic study of *Quercus langbianensis* complex based on morphology, and DNA barcodes of classic and next generation sequences.

Abstract

We revised the taxonomy of *Quercus langbianensis* and its relatives in Vietnam and Cambodia based on evidence obtained from field observations, morphological comparison of herbarium specimens, and molecular analysis using both classic and next generation DNA markers. Based on Bayesian phylogeny using *rbc*L*, mat*K and ITS regions and Neighbor-joining tree using genome-wide sequences amplified with multiplexed inter-simple sequence repeat (ISSR) primers (MIG-seq), we recognized ten species in the complex in Vietnam and Cambodia, three of which are newly described in this paper: *Q. baolamensis*, sp. nov.*, Q. bidoupensis*, sp. nov., and *Q. honbaensis*, sp. nov. These new species are all phenotypically similar to *Q. langbianensis s.str.* in having lanceolate to oblanceolate leaf shape, upper 4–5/6-serrated leaf margin, acute or acuminate leaf apex, and bracts of cupule arranged in 5–9 rings but distinguished both morphologically and phylogenetically. In molecular phylogenetic reconstructions, *Q. bidoupensis* is not close to any other species. In the Bayesian tree, *Q. honbaensis* is sister to both *Q. blaoensis* and *Q. camusiae* that are found in the same locality but morphologically distinct, and those three species are sister to *Q. langbianensis s.str*., while *Quercus baolamensis* is not sister to *Q. langbianensis s.str* in both the Bayesian tree and MIG-seq tree. In addition, we recognized *Q. cambodiensis* and *Q. baniensis* previously reduced to *Q. langbianensis s. lat.* as distinct species. Six species were in need of lectotypification and that is undertaken herein.

Keywords. DNA barcoding, Fagaceae, MIG-seq*, Quercus*, taxonomy, Vietnam.

Introduction

The genus *Quercus* L., with 400–500 species, is the largest genus among the family Fagaceae (Nixon 1993, Valencia-A et al. 2016). The genus is widely distributed in the northern Hemisphere including tropical montane forests in South East Asia and often dominant in temperate deciduous forests in East Asia, Europe and North America, and desert scrubs in the Mediterranean (Nixon 1993, Hubert et al. 2014, Valencia-A et al. 2016). In Vietnam, 45 species of the genus *Quercus* have been recognized (Ho 2003, Ban 2005, Binh et al. 2018c) but taxonomic identities

of some species remain to be revised. One of them is *Quercus langbianensis* Hickel & A.Camus (1921), described from Mt. Langbian of Lam Dong Province, southern Vietnam. Following previous studies including Deng et al. (2010), The Plant List (2013) adopted a broad concept of this species by treating the following seven names as synonyms: *Q. baniensis* A.Camus, *Q. blaoensis* A.Camus, *Q. camusiae* Trel. ex Hickel & A.Camus (a replacement name of *Q. geminata* Hickel & A.Camus), *Q. dilacerata* Hickel & A.Camus, and *Q. donnaiensis* A.Camus from Vietnam, *Q. cambodiensis* Hickel & A.Camus from Cambodia and *Cyclobalanopsis faadooensis* Hu from mainland of China. However, our recent comparison based on the collections of *Q. camusiae* and *Q. cambodiensis* from their type localities revealed that both *Q. camusiae* and *Q. cambodiensis* are distinct species from *Q. langbianensis s.str*. This finding triggered us to reexamine the taxonomy of *Q. langbianensis s. lat.* hereafter designated as "*Q. langbianensis* complex", and its similar species such as *Q. auricoma* A.Camus in which *Q. cambodiensis* was recently included (Tagane et al. 2017). Deng et al. (2010) studied the relationship of *Q. camusiae, Q. cambodiensis* and *Q. langbianensis* and concluded that the three species are phenotypically indistinguishable. However, their study was based on the comparison of a limited number of herbarium specimens.

In this study, we observed and collected specimens of the *Q. langbianensis* complex more widely: Mt. Hon Ba of Khanh Hoa Province (the type locality of *Q. camusiae*), some localities of Lam Dong Province (near the type locality of *Q. langbianensis s.str.*), Mt. Ba Na (the type locality of *Q. baniensis*) and Mt. Bokor of Cambodia (the type locality of *Q. cambodiensis*). In Mt. Hon Ba, *Q. camusiae* was found in the higher elevation whereas two additional morphologically similar but distinct species were found in the lower elevation. Our observations in the field revealed that two neighboring provinces of southern Vietnam, Khanh Hoa Province and Lam Dong Province, harbor the highest diversity of the *Q. langbianensis* complex including three unknown species. However, those species are phenotypically very similar to each other and evidence based on molecular analysis is needed to elucidate their identities and relationships.

Recently, molecular studies of the genus *Quercus* have succeeded in elucidating phylogenetic relationships within the genus by using multiple gene markers (Hubert et al. 2014, Simeone et al. 2016) or RAD-seq (Hipp et al. 2014, Cavender-Bares et al. 2015, Fitz-Gibbon et al. 2017). In this study, we employ both classic multiple gene markers (*rbc*L, *mat*K and ITS) and genome-wide markers using the next generation sequencing platform (MIG-seq; Suyama and

Matsuki 2015) to clarify relationships of the species within *Q. langbianensis* complex. As in RAD-seq, MIG-seq provides genetic markers of relatively short sequence reads determined by the next generation sequencer, but it is obtained with a PCR-based procedure without restriction enzyme digestion steps and widely applicable to field samples even with low-quality DNA and/or small quantities of DNA (Suyama and Matsuki 2015).

The purpose of this paper is to revise taxonomy of the *Q. langbianensis* complex based on evidence obtained from field observations, morphological studies and molecular data from both classic and next generation DNA markers. In conclusion, we distinguished 10 species in the *Q. langbianensis* complex, including the seven species treated as synonyms of *Q. langbianensis* (Plant List 2013) and the remaining three undescribed species. We describe them as *Q. baolamensis*, sp. nov., *Q. bidoupensis* sp. nov. and *Q. honbaensis* sp. nov.

Materials and methods

Observations and collections in the field

The field survey was carried out in 13 conservation areas (national parks, nature reserves and conservation area) in Vietnam and one national park in Cambodia (Fig. 1). In Hon Ba Nature Reserve, Khanh Hoa Province, southern Vietnam, we placed eight rectangular plots of 100 m × 5 m at various locations from 225 m to 1,498 m altitude and recorded girth and height for all the individual trees above 4 m tall within the plots (Table 1). We recorded trees in the same way for the following localities: two plots at 1,553 m and 1,807 m in Bidoup-Nui Ba National Park, Lam Dong Province; three plots at 1,850 m, 2,225 m and 2,933 m in Hoang Lien National Park (Mt. Fan Si Pan), Lao Cai Province; four plots at 86 m, 650 m, 1,420 m and 1,720 m in Vu Quang National Park, Ha Tinh Province; two plots at 450 m and 1,274 m in Bach Ma National Park, Thua Thien Hue Province; and three plots at 833 m, 1,070 m and 1,376 m in Ngoc Linh Nature Reserve, Kon Tum Province. In those localities, we also made general collections of vascular plants outside of the plots, with particular attention to the species of Fagaceae. In addition to the above 6 conservation areas, we made general sampling of Fagaceae in the following 6 conservation areas: Ba Na Nature Reverse, Da Nang Province; Ba Vi National Park, Ha Noi Capital; Cuc Phuong National Park, Ninh Binh Province; Dong Nai Nature Reserve, Dong Nai Province; Pu Mat National Park, Nghe An Province; Son Tra Conservation Area; and Cao Vit Gibbon Conservation Area, Cao Bang Province. In Mt. Bokor, Cambodia, 20 plots from 266 m to 1,048 m altitude were

established and *Q. cambodiensis* was sampled (Zhang et al. 2016, Tagane et al. 2017). For each specimen we collected, we took photos in the field and gathered samples of silica gel-dried leaf pieces for DNA isolation.

Among the collections of *Quercus*, we regarded species having the following traits as members of the *Q. langbianensis* complex: mature leaves are 12–17 cm long, 3–5 cm wide, serrated along the upper 5/6 to 1/3 margin (although young leaves of *Q. camusiae* are often almost entire), acute or acuminate at apex, cuneate at base and hairy when young but almost glabrous when mature; cupule obconical or bowl- or cup-shaped, bracts of cupule arranged in 5–9 rings, and covers 1/4 to 2/3 of a nut that is ovoid or subglobose to ellipsoid. We do not include *Q. auricoma* in the *Q. langbianensis* complex because mature leaves have entire margin and smaller size (5.5–7 cm long, 2–2.7 cm wide, from *E. Poilanei 13098* (P)).

In this study, 46 samples including 9 species of the *Quercus langbianensis* complex (*Q. baniensis, Q. baolamensis, Q. bidoupensis, Q. blaoensis, Q. cambodiensis, Q. camusiae, Q. donnaiensis, Q. honbaensis, Q. langbianensis s.str.*) and ten species of non-*Quercus langbiangensis* complex (*Q. annulata, Q. auricoma, Q. austrocochinchinensis, Q. braianensis, Q. dijiringensis, Q. helferiana, Q. kerrii, Q. macrocalyx, Q. neglecta,* and *Q. poilanei*) were used for morphological and DNA studies. One species of *Trigonobalanus*, *T. verticillatus* Forman was also analyzed as an outgroup in phylogenetic analysis. Three to four sets of voucher specimens were collected from each locality and deposited in FU and herbaria of each protected area, DLU and VNM.

DNA extraction

DNA was isolated from each silica-gel dried sample by the CTAB method (Doyle and Doyle 1987) with the following modifications: dried leaf material was milled by QIAGEN TissueLyser to obtain fine powder and washed three times in a 1 ml buffer (including 0.1 M HEPES, pH 8.0; 2% Mercaptoethanol; 1% PVP; 0.05 M Ascorbic acid) as in Toyama et al. (2015).

Classic DNA sequencing

DNA regions of the large subunit of ribulose-1,5-biphosphat carboxylase oxygenase (*rbc*L), maturase K (*mat*K) and the internal transcribed spacer (ITS) were amplified with the following primer sets (sequence: 5' to 3'): *rbc*La-F (ATGTCACCACAAACAGAGACTAAAGC,

Levin et al. 2003), *rbc*L-724r (TCGCATGTACCTGCAGTAGC, Fay et al. 1997); *mat*K-XF (TAATTTACGATCAATTCATTC, Ford et al. 2009), *mat*K-1326R (TCTAGCACACGAAAGTCGAAGT, Cuénoud et al. 2002); ITS-18F (GTCCACTGAACCTTATCATTTAGAGG, Rohwer et al. 2009), ITS-26R (GCCGTTACTAAGGGAATCCTTGTTAG, Rohwer et al. 2009). The sequences of *rbc*L, *mat*K and ITS were amplified with Tks GflexTM DNA Polymerase (Takara Bio, Kusatsu, Japan) following the protocols of Kress et al. (2009), Dunning and Savolainen (2010) and Rohwer et al. (2009), respectively. The PCR product was cleaned with 0.5 µl of ExoSap-IT enzyme (GE Healthcare, Little Chalfont, UK) and 1.5 µl of distilled water and incubated at 37 °C for 30 min and subsequently at 80 °C for 15 min for deactivation of the enzyme. Sequence reactions were proceeded using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The reaction mixtures were analyzed in an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Next generation DNA sequencing – MIG-seq

For 105 samples, we amplified thousands of short sequences (loci) from each genome using primers designed for "multiplexed ISSR genotyping by sequencing" (MIG-seq, Suyama and Matsuki 2015) and used presence/absence of each sequence (amplicon) in each sample for phylogenetic tree reconstruction regardless of whether it has SNP or not, as sequence-based dominant markers. The experimental standard conditions were performed following Suyama and Matsuki (2015). The 1st PCR step was performed to amplify ISSR regions from genomic DNA with MIG-seq primer set-1. The products of the 1st PCR were diluted 50 times for each 1st PCR product with deionized water. The 2nd PCR step was conducted independently to add individual indices to each sample using indexed primers. Then, 3 µl of each 2nd PCR product was pooled as a single mixture library. The mixture was purified, and fragments in the size range 350–800 bp were selected by a Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA). Finally, the concentration of size-selected library was measured by using a SYBR green quantitative PCR assay (Library Quantification Kit; Clontech Laboratories, Mountain View, CA, USA) with approximately 10 pM of libraries that were used for sequencing on an Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 cycle, Illumina).

Phylogenetic analyses

In classical phylogenetic analyses, we constructed a phylogenetic tree combining nucleotide sequences of the three DNA regions comprising *rbc*L, *mat*K and ITS for 30 samples of 29 *Quercus* species and one *Trigonobalanus verticillatus* (as an outgroup). All DNA sequences were newly generated in this study. The sequences were aligned by MEGA v7.0 (Kumar et al. 2016). For reconstructing phylogeny, we used a Bayesian method implemented in the program BEAST v1.8.4 (Drummond et al. 2012). The GTR + γ model of molecular evolution and an uncorrelated lognormal (UCLN) relaxed-clock model were selected to infer relative divergence times. In computation, the program was started with a random tree, and a tree prior that was useful for species-level was set according to a Yule process (Drummond and Rambaut 2007). We ran five independent chains of 100 million generations each, sampling every 10,000 generations. The first 1,000 trees were discarded as burn-in from each run. The remaining trees from each run were combined by using LogCombiner v 1.6.1 (Drummond and Rambaut 2007). Among the posterior distribution of 9,000 trees, the maximum clade credibility tree was identified using TreeAnnotator v 1.6.1 (Drummond and Rambaut 2007) with a posterior probability limit of 0.5 and median node heights. The congruence among *rbc*L, *mat*K and ITS trees was tested using incongruence length difference test (Farris et al. 1994) implemented in PAUP* 4.0b10 (Swofford 2003). Because the incongruence was rejected (p=0.06), we constructed a combined tree using concatenated sequences.

For MIG-seq, we pretreated raw data from 105 samples and completed quality control following Suyama and Matsuki (2015). We used the program 'fastx_trimmer' in the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) to trim read 2 sequences including 12 bases of SSR region and two bases of anchor sequences in the 1st primers. We used option 'quality_ filter' of FASTX-Toolkit to select reads in which 40% or more sequences had quality scores Q30 or more. Then we used TagDust program (Lassmann et al. 2009) to remove the reads derived from extremely short library entries and trimmed read 1 and read 2 sequences. Then, we assembled loci from the quality-filtered reads data with the *de novo* map pipelines (ustacks, cstacks, sstacks) in Stacks software package version 1.35 (Catchen et al. 2011) and then prepared a table of presence/absence of loci in each individual from the outputs of the populations pipeline of Stacks 1.35. Using ustacks, we assembled homologous sequences (loci) in each individual with the following settings: minimum depth of coverage (m) = 10, maximum distance allowed between stacks (M) = 1, maximum distance allowed to align secondary reads to primary stacks (N) = 1, and

maximum gaps = 2. Using cstacks, we built a catalog of consensus loci for all the individuals by assembling loci in each individual assembled using ustacks, with the number of mismatches allowed between sample loci (n) = 2. Using sstacks, we associated IDs of loci in each individual with IDs of the consensus loci. Finally, we determined presence/absence of loci in each individual from a haplotypes list obtained using the populations pipeline. The populations pipeline output file haplotypes.tsv provides genotypes of individuals at each locus. For each individual, we recorded a locus that had genotype information as "1" and a locus that had no genotype information as "0". We obtained a list of loci that were detected at least in one individual (1/105 = 0.01) with following settings: all samples belong to the same population, and threshold frequency of haplotype count in a population (r) = 0.001, a threshold one-order higher than 0.01. Using presence/absence (1/0) data of loci, we computed distance matrix, constructed a neighbor-joining (NJ) tree, and examined the reliability of tree topology by bootstrapping with 1000 replicate using PHYLIP ver. 3.695 (Shimada and Nishida 2017) as follows; 1000 times resampling with Seqboot, distance computation with Restdist, tree construction with Neighbor and consensus tree construction with Censense. The resulted tree was visualized with FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). We made a phylogenetic analysis first for 105 samples including more *Quercus* species and then reduced the sample size to 31 by focusing on the *Q. langbianensis* complex. A total of 16,809 loci were used for the final phylogenetic tree.

Morphological and taxonomic comparison

Our collections contain considerable numbers of sterile specimens including those from young trees that are often morphologically different from adult trees. Thus, after we obtained phylogenetic trees, we carefully reexamined morphological traits of leaves and shoots as well as reproductive organs if available, and distinguished species. If two OTUs are morphologically distinguishable and also not monophyletic on phylogenetic trees, we regarded them as two distinct species. Then, we identified them by a thorough literature review and comparisons with type specimen images available online (e.g. JSTOR Global Plants, http://plants.jstor.org/). In *Q. langbianensis* complex, lectotypification was needed for *Q. baniensis, Q. blaoensis, Q. cambodiensis, Q. camusiae, Q. dilacerata* and *Q. donnaiensis*. One of our co-authors, J.S. Strijk examined specimens at P for lectotypification; for each species, selected was one of the specimens cited in the original description, which best represents the diagnostic traits of each species.

Results

Observation in the field

In Hon Ba Nature Reverse, we examined tree diversity in eight plots of 100 m × 5 m and found four species of *Quercus* (Table 1) including *Q. poilanei* and three species of the *Q. langbianensis* complex: *Q. blaoensis*, *Q. camusiae* and an undescribed species, *Q. honbaensis*. *Quercus camusiae* was found in the two plots at 1,336 m and 1,498 m altitude and one of canopy trees in the latter. *Quercus honbaensis* was found in three plots at 225 m, 400 m, and 617 m altitude, and occurred sympatrically with *Q. blaoensis* in the plot at 225 m altitude. *Quercus honbaensis* was one of the canopy trees at both 225 m and 400 m altitude (Table 1). In late February of 2014, *Q. honbaensis* had mature fruits and *Q. blaoensis* had young fruits. Two species were distinct in pubescence on young shoots (*Q. honbaensis* has long, very thin and curly hairs vs. *Q. blaoensis* has short, thicker and straight hairs). *Quercus camusiae* was distinct from *Q. honbaensis* and *Q. blaoensis* in that shoots and leaves were golden tomentose when young.

In Bidoup-Nui Ba National Park, approximately 100 km west of Mt. Hon Ba, we examined tree diversity in two plots at 1,553 m and 1,807 m altitude, and we found *Q. langbianensis s. str.* at 1,553 m altitude. *Quercus langbianensis s. str.* was similar to *Q. camusiae* in having golden tomentose cupules, but different in distinctly toothed leaves and longer nuts (vs. almost entire or with only a few low teeth in *Q. camusiae*). We surveyed the flora above 800 m altitude in Bidoup-Nui Ba National Park and did not find any of *Q. camusiae* and *Q. honbaensis.* On the other hand, we found two additional and unknown species of the *Q. langbianensis* complex: *Q. bidoupensis* and *Q. donnaiensis*. *Quercus bidoupensis* was distinct from *Q. langbianensis s. str.* in having oblong-lanceolate leaves, acuminate and slightly caudate at apex, and undulate and distinctly serrate along the upper half of margin. *Quercus donnaiensis* was similar to *Q. bidoupensis* in leaf shape but differs in its margin not undulate, serrated only near the apex and with 3–5 teeth. In our general collection in Lam Dong Province, we collected three species of the *Q. langbianensis* complex: *Q. bidoupensis* and *Q. donnaiensis* in Lam Tranh District, and another undescribed species, *Q. baolamensis,* in Bao Lam District.

In Ba Na Nature Reverse and Son Tra Natural Conservation Area, central Vietnam, we found *Q. baniensis* of the *Q. langbianensis* complex and *Q. poilanei* and *Q. auricoma* of non-*Q. langbianensis* complex.

In the top plateau of Mt. Bokor, Cambodia, we collected *Q. cambodiensis* of the *Q. langbianensis* complex and *Q. augustinii* of non-*Q. langbianensis* complex.

*A phylogenetic tree combining three DNA regions (rbc*L*, mat*K*,* and ITS*)*

A total of 2,034 bases combined of three DNA regions (657 bp for *rbc*L*,* 834 bp for *mat*K and 543 bp for ITS) included 142 variable sites, among which 56 bases was parsimony-informative (Table 2). According to the Bayesian tree combining the three regions (Fig. 2) two major clades were supported by posterior probabilities higher than 80%: Clade 1 with 85% posterior probability consists of five species of non-*Quercus langbianensis* complex (*Q. poilanei, Q. kerrii, Q. austrocochinchinensis, Q. helferiana* and *Q. braianensis*) and Clade 2 with 82% posterior probability includes seven species of the *Q. langbianensis* complex (*Q. cambodiensis* and six Vietnamese species) and five species of non-*Quercus langbianensis* complex (*Q. neglecta* nested with the *Q. langbianensis* complex and *Q. annulata, Q. auricoma, Q. djiringensis* and *Q. macrocalyx*). In Clade 2, *Q. cambodiensis* was sister to *Q. neglecta* with 81% posterior probability and clearly separated from the Vietnamese species of the *Q. langbianensis* complex (*Q. langbianensis s. str., Q. baniensis, Q. blaoensis, Q. honbaensis, Q. baolamensis* and *Q. camusiae*). *Quercus langbianensis s. str.* was sister to *Q. blaoensis, Q. camusiae* and *Q. honbaensis* with a strong branch support (PP = 1.00). *Quercus camusiae* was sister to *Q. blaoensis* with a high branch support (PP = 0.99). *Quercus baolamensis* and *Q. baniensis* were clustered together, but with weak branch support (PP = 0.64).

Trees based on single gene sequences gave lower resolution but ITS tree (see supplementary Figure S1) supported the following points. (1) A clade consisting of seven species of the *Q. langbianensis* complex and *Q. neglecta* was supported by 100% PP. (2) *Quercus bidoupensis* was not clustered with the other seven species of the *Q. langbianensis* complex. (3) A clade including five species of non-*Q. langbianensis* complex was supported by 70% PP. In cpDNA tree (see supplementary Figure S2), neither the seven species of the *Q. langbianensis* complex nor the five species of non-*Q. langbianensis* complex was monophyletic. Neither of *Q. poilanei*, *Q. austrocochinchinensis*, *Q. helferiana* and *Q. braianensis* was monophyletic whereas those four species were monophyletic in ITS tree.

A phylogenetic tree using MIG-seq

A neighbor-joining (NJ) tree based on MIG-seq for 31 samples of *Quercus* recognized three major clades excluding an outgroup of *Trigonobalanus* (Fig. 3). Clade M1 includes single species, *Q. bidoupensis*. Clade M2 with a 100% bootstrap value consists of five species of non-*Q. langbianensis* complex (*Q. poilanei, Q. kerrii, Q. austrocochinchinensis, Q. helferiana* and *Q. braianensis*). Clade M3 with 100% bootstrap value includes *Q. neglecta, Q. macrocalyx, Q. auricoma* and eight species of the *Q. langbianensis* complex. Within this clade, *Q. cambodiensis* was sister to *Q. neglecta* with a 74% bootstrap value. *Quercus honbaensis* and *Q. baolamensis* were monophyletic with a bootstrap value of 100%. *Quercus donnaienis* and *Q. camusiae* were also monophyletic with a bootstrap value of 75%. *Quercus blaoensis* and *Q. langbianensis s. str.* of the *Q. langbianensis* complex are clustered with *Q. baniensis, Q. auricoma, Q. macrocalyx,* forming a clade with 82% bootstrap value.

Discussion

The results of tree gene tree (Bayesian tree) and MIG-seq tree (NJ tree) were mostly consistent. First, five species of non-*Q. langbianensis* complex (*Q. poilanei, Q. kerrii, Q. austrocochinchinensis, Q. helferiana* and *Q. braianensis*) formed a highly supported clade, Clade 1 or Clade M2. This clade was supported also in ITS tree. Second, three gene and MIG-seq trees matched in Clade 2 and Clade M3. Third, the species of the *Q. langbianensis* complex except *Q. bidoupensis* formed a highly supported clade (also in ITS tree) and *Q. auricoma, Q. macrocalyx* and *Q. neglecta* of non-*Q. langbianensis* complex were included in this clade (Fig. 4). Fourth, *Q. cambodiensis* was sister to *Q. neglecta* and separated from the Vietnamese species of the *Q. langbianensis* complex by relatively high supports (posterior probability 0.81 and bootstrap probability 74%). Fifth, *Q. bidoupensis* was placed in Clade 2 or Clade M1 and not close to the other species of the *Q. langbianensis* complex (also in ITS tree). The cpDNA tree did not support monophilies of *Q. poilanei, Q. austrocochinchinensis, Q. helferiana* and *Q. braianensis* that were monophyletic in ITS tree, three gene tree and MIG-seq tree and thus cpDNA tree alone provides less reliable evidence.

The consistent topology of three gene and MIG-seq trees (Fig. 2–4) provided reliable evidence to resolve taxonomy of "species" currently treated as synonyms of *Q. langbianensis s. lat.* (*Q. camusiae, Q. blaoensis, Q. cambodiensis* and *Q. baniensis*). First, *Q. cambodiensis* is separated as a species because it is sister to *Q. neglecta* that is morphologically distinct in linear

leaves and small nuts and has been treated as a distinct species in the Flora of China (Huang et al. 1999 as *Cyclobalanopsis neglecta*). Among the others, both *Q. camusiae* and *Q. blaoensis* are native in Hon Ba Nature Reserve where *Q. camusiae* occurs in the higher elevation and *Q. blaoensis* occurs in the lower elevation (Table 1). Because *Q. camusiae* and *Q. blaoensis* are sister to each other in the MIG-seq tree and not sympatric but paratactic in the distribution, those can be treated as two infraspecific taxa (varieties or subspecies) or two different species. Considering morphological distinction described above, we adopt the latter treatment. *Quercus blaoensis* co-occurs with another undescribed species: *Q. honbaensis*. Because *Q. blaoensis* and *Q. honbaensis* are sympatric and morphologically distinct, those are recognized as different species. The monophyly of *Q. honbaensis* and *Q. baolamensis* was strongly supported in the MIG-seq tree with a bootstrap value of 100%. While *Q. honbaensis* occurs in the elevation lower than 617 m of Hon Ba Nature Reserve, Khanh Hoa Province, *Q. baolamensis* is collected at 1000 m of the Lam Dong province. Considering this distinction in cupule and nut morphology (Fig. 6 and 12), we treat them as two distinct species. While *Q. baniensis* is found in Da Nang Province of the central Vietnam, the other five species (*Q. baolamensis, Q. blaoensis, Q. camusiae, Q. honbaensis* and *Q. langbianensis s. str.*) occur in Khanh Hoa or Lam Dong Province of the southern Vietnam. From phylogenetic trees and morphological observations, it is difficult to relate *Q. baniensis* with any of the five species. In particular, a sister relationship between *Q. langbianensis s. str.* and *Q. baniensis* is not strongly supported (Fig. 4). Thus, we treat *Q. baniensis* as a species.

Although the topologies of the three gene and MIG-seq tree are mostly consistent, there are some notable differences, particularly in Clade 2 and Clade M3 containing the *Q. langbianensis* complex (Fig. 4). In the Bayesian tree based on the three regions of *rbc*L, *mat*K and ITS, the monophyly of the Vietnamese species of the *Q. langbianensis* complex was only weakly supported (PP = 0.28), whereas it was strongly supported in MIG-seq tree (bootstrap value 100%). This higher support in MIG-seq tree was obtained because MIG-seq provided more informative sites for constructing phylogenetic relationships among the species in the *Q. langbianensis* complex. *Quercus auricoma* and *Q. macrocalyx* were included in a clade of the *langbianensis* complex in MIG-seq tree but clustered with *Q. annulata* in Bayesian tree. Further studies using more gene markers are needed to derive a conclusion on the placement of these two species.

Comparison based on morphological characters both in the field and dried specimens of the herbarium and the molecular evidence for the *Q. langbianensis* complex revealed that *Q.*

baniensis, Q, blaoensis, Q. cambodiensis, Q. camusiae, and *Q langbianensis s. str.* are all distinct species (Table 3). In addition, we conclude that three species among the *Q. langbianensis* complex are undescribed and below we describe them as *Q. baolamensis*, *Q. bidoupensis* and *Q. honbaensis*. In our study, we collected only sterile specimens of *Q. donnaiensis* and we could not determine the sequence of ITS for *Q. donnaienis* due to low DNA quality. In MIG-seq tree, *Q. donnaienis* and *Q. camusiae* were monophyletic with a bootstrap value of 64%. In vegetative traits, *Q. donnaiensis* is distinguished from *Q. camusiae* in having distinct serrations on upper 1/3 of leaf margin (vs. almost entire in *Q. camusiae*). We need to examine additional materials having fruits to conclude whether those two are distinct species or infraspecific taxa. In the following taxonomic section, we treat them as two species tentatively. Among species treated as synonyms of *Q. langbianensis s. lat.* (Plant List 2013)*, Q. dilacerata,* is morphologically distinct as described in the following taxonomic section, but we could not obtain DNA samples of this species. Further studies using phylogenetic analysis are required to clarify the identity of *Q. dilacerata*.

While *Q. cambodiensis* is treated as a synonym of *Q. auricoma* by Tagane et al. (2017), those two species are not sister to each other in both Bayesian and MIG-seq trees. The treatment of Tagane et al. (2017) is based on the broad concept of *Q. auricoma* adopted in the Flora of Thailand (Phengklai 2008) in which a species morphologically similar to *Q. cambodiensis* in northern and northeastern Thailand is treated as *Q. auricoma*. However, after examining our collections of *Q. auricoma* from Son Tra (*V3135, V3138*) that is morphologically identical with the species of the type specimen of *Q. auricoma*, we concluded that the species treated as *Q. auricoma* in the Flora of Thailand (Phengklai 2008) is different from genuine *Q. auricoma,* in that leaves are serrate along the upper 1/2–1/3 margin (vs. completely entire in *Q. auricoma*), nuts ovoid to oblong (vs. suborbicular), and cupules densely hairy (vs. less hairy). As far as we know, *Q. auricoma* is endemic to Vietnam. Further studies are needed to elucidate identity of the species called "*Q. auricoma*" in Thailand.

Key to the species of *Quercus langbianensis* complex in Vietnam and Cambodia

1a. Leaves undulate, distinctly serrate in the upper 1/2. Cupules obconical, enclosing 1/3 of the nut, bracts set in 5–6 rings, margin entire. Nut ovoid, scar convex..... ***Q. bidoupensis***

1b. Leaves not undulate..... **2**

2a. Leaves almost entire or with a few low teeth. Cupule cup-shaped, enclosing <1/2 of nut, bracts set in 6–8 rings, margin undulate at least in the lower rings. Nut subglobose, scar convex or flat.**3**

2b. Leaves distinctly serrate in the upper 5/6 to 1/3 of margin…….. **4**

3a. Cupule distinctly narrowed at base, bracts set in 6 rings, sparsely dissected in the lower rings. Nut scar convex..... ***Q. camusiae***

3b. Cupule not distinctly narrowed at base, bracts set in 7–8 rings, margin distinctly toothed in two lower rings. Nut scar flat..... ***Q. cambodiensis***

4a. Cupule obconical **5**

4b. Cupule cup-shaped or bowl-shaped.....**6**

5a. Margin distinctly serrate in the upper 1/3; secondary veins 7–11 pairs; petioles 1.2–2(–2.9) cm long; Cupules enclosing 2/3 of the nut. Nut ovoid ..... ***Q. baniensis***

5b. Margin distinctly serrate in the upper 5/6–3/4(–2/3); secondary veins (9–)10–14(–16) pairs; petioles 0.8–1 cm long. Cupules enclosing 1/3–1/2 of the nut. Nut obovoid to ellipsoid..... ……..***Q. honbaensis***

6a. Cupule bowl-shaped, enclosing about 2/3 of nut, bracts set in 7 rings, bract margin distinctly toothed in all rings. Nut subglobose ..... ***Q. dilacerata***

6b. Cupule cup-shaped, enclosing 1/3–2/3 of nut, bracts set in 5–9 rings, bract margin nearly entire… **7**

7a. Cupules enclosing 2/3 of the nut. Nut ovoid. Young shoots covered with straight whitish hairy. Leaves distinctly serrate in the upper 1/3 ..... ***Q. blaoensis***

7b. Cupules enclosing 1/3–1/2 of the nut. Nut subglobose, ovoid-ellipsoid, obovoid to ellipsoid. Young shoots covered with golden tomenose or almost glabrous. Leaves regularly distinctly serrate in the upper 1/3–1/2.....**8**

8a. Cupules enclosing 1/2 of the nut, bracts set in 5–6 rings. Leaves regularly distinctly serrate in the upper 1/3. Nut subglobose, scar convex .....***Q. donnaiensis***

8b. Cupules enclosing 1/3 of the nut, bracts set in 6–9 rings. Leaves regularly distinctly serrate in the upper 1/2 or upper 1/3. Nut scar flat or convex **9**

9a. Leave regularly distinctly serrate in the upper 1/2; petiole 0.4–1 cm long. Nut ovoid-ellipsoid, scar flat, sparsely hairy ..... ***Q. baolamensis***

9b. Leave regularly distinctly serrate in the upper 1/3; petiole 1–2 cm long. Nut obovoid to ellipsoid, scar convex, densely hairy .....***Q. langbianensis s. str.***

Taxonomic treatments of *Quercus langbianensis* complex in Vietnam and Cambodia

Quercus baniensis A.Camus, Chênes Atlas 2: 123, pl. 231 (1935-1936), nom. nud.; Bull. Soc. Bot. France 83: 343 (1936).

Fig. 2.5

Type: VIETNAM. "Mont Bani, in the main coast range about 25 kilometers from Tourane", 4–13 June, 1927, *J. & M.S. Clemens 3455* (lectotype: P [P00753998!]; isolectotype: BM [BM000839274, BM000839274, image!], MICH [MICH1210512, image!], U [U0238780, image!], US [US00089422, image!], designated here).

Distribution and habitat: VIETNAM. Da Nang Province: Ba Na Nature Reserve. In this study, this species was found along roadside and edge of evergreen forest, at 707 and 789 m altitude.

Additional specimens examined: VIETNAM. Ba Na Nature Reserve, 16°00'07.30"N, 108°01'33.90"E, alt. 707 m, 29 May 2015, *Tagane S., Toyama H., Nguyen N., Nguyen C. V3089* [young fr.] (DLU, FU); ibid., 16°00'10.0"N, 108°02'17.8"E, alt. 789 m, 19 Feb. 2017, *Hoang T.S. & Tagane S. V6922* (DLU, FU).

Note: Camus (1935) illustrated *Quercus baniensis* in Chênes Atlas 2 (Pl.231), and later Camus (1936) effectively described this species based on the specimen *Clemens 3455* collected from mountain Bani, Vietnam. We examined six specimens of *Clemens 3455* in P, BM, MICH, U and US directly or using digitized images on the web. Among them, we selected *Clemens 3455* in P [P00753998] as lectotype of *Q. baniensis* because the trait of a nut is well represented in this specimen.

Quercus baolamensis Binh & Ngoc, **sp. nov.**

Fig. 2.6

Diagnosis: Quercus baolamensis is most similar to *Q. langbianensis s. str.,* but differs in having leaf margin regularly distinctly serrate in the upper 1/2 (vs. serrate in the upper 1/3) and shorter petioles 0.4–1 cm long (vs. 1–1.8 cm long).

Type: VIETNAM. Lam Dong Province: Bao Lam District, B40 Pass, roadside and edge of evergreen forest, 11°43'37"N, 107°42'34.5"E, alt. 1,000 m, 13 June 2015, with fruits, *Ngoc N.V., Binh H.T., Dung L.V., Truong N.K. V3191* (holotype: KYO!; isotypes: FU!).

Description: Tree, 6–8 m tall. Young twigs almost glabrous except near bud, 1–1.2 mm in diam., sometimes sulcate. Old twigs glabrous, brownish black when dry, lenticellate. Stipules linear, 3–

5 mm long, hairy on both surfaces, caducous. Leaf blades elliptic to elliptic-lanceolate, or rarely oblanceolate, (5.2–)9–15 × 1.7–4.5 cm, thinly coriaceous, glossy adaxially, pale green abaxially, acuminate at apex, cuneate at base, margin regularly distinctly serrate in the upper 1/2, having 9–12 teeth per side, glabrous on both surfaces; midrib slightly prominent adaxially, prominent abaxially, lateral veins (7–)10–13 pairs, straight and running into the teeth of margin, slightly prominent adaxially, prominent abaxially, at angle of 40–45 degree from midrib, tertiary veins scalariform-reticulate, visible on both surfaces; petioles 0.4–1 cm long, whitish hairy when young, glabrescent. Male and female inflorescences not seen. Infructescences axillary or terminal, erect spike, rachis 0.5–1.4 cm long, 1–3 mm in diam., tomentose when young, glabrescent when mature. Mature fruits ca. 2.9 cm high (including cupule), usually 1–(2) per infructescence, sessile; cupules obconical, 1.2 cm high, 1.5 cm in diam., enclosing 1/3 of the nut, wall comprising bracts, arranged in 7 rings, margin of rings nearly entire; nut ovoid-ellipsoid, 2.5 cm high, 1.5 cm in diam., apex nearly flat, sparsely hairy except densely appressed hairy around stylopodia, stylopodia up to 4 mm long, basal scar flat, 0.8 cm in diam., glabrous.

Phenology: Fruiting specimens were collected in June.

Distribution and habitat: VIETNAM. Lam Dong Province: Bao Lam District. At present, this species is known only from the type locality. We found only one individual along roadside and edge of evergreen forest, at 1,000 m altitude.

Etymology: The specific epithet is derived from the name of its type locality, Bao Lam District.

Quercus bidoupensis Binh & Ngoc, **sp. nov.**

Fig. 2.7

Diagnosis: Similar to *Quercus langbianensis s. str.* in leaf shape, the number of secondary veins, and basal scar of the nut convex, but distinguished in having bud oblong to ellipsoid (vs. globose to broadly ovoid), undulate and distinctly serrate leaf margin along the upper half (vs. regularly distinctly serrate in the upper 1/3), obconical cupules (vs. cup-shape) and bracts of cupule arranged in 5–6 rings (vs. 6–9 rings), nut ovoid (vs. obovoid to ellipsoid).

Type: VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park, hill evergreen forest dominated by the species of Fagaceae, 12°09'52.95"N, 108°32'00.38"E, alt. 1,698 m, 24 Feb. 2016, *Tagane S.*, *Toyama H., Nagamasu H., Naiki A., Dang Son, Nguyen V. Ngoc, Wai J. V4328* (holotype: KYO!; isotypes: DLU!, the herbarium of Bidoup-Nui Ba National Park).

Description: Tree, 8 m tall. Buds oblong to ellipsoid, ca. 2–4 mm high, ca. 1–2 mm in diam., scales 6–7 rows, imbricate, ovate-triangular, ca. 3 × 2.5 mm, apex obtuse, margin ciliate, densely hairy or glabrous outside, glabrous inside. Twigs grayish, glabrous, lenticellate. Leaf blades oblong-lanceolate, (7.5–)10–13 × 2.5–4 cm, thinly coriaceous, blackish brown adaxially, pale brown abaxially when dry, glabrous on both surfaces, acuminate at apex, cuneate at base, margin undulate, distinctly serrate in the upper 1/2; midrib sunken adaxially, prominent abaxially, lateral veins 10–13 pairs, slightly prominent adaxially, prominent abaxially, at angle of 45–50 degree from midrib, and running into the teeth of margin, tertiary veins scalariform-reticulate, slightly prominent, visible on both surfaces; petioles 1.3–2 cm long, blackish when dry, glabrous. Male and female inflorescences and infructescences not seen. Fruits 2.6 cm high (including cupule); cupules obconical, 1.3–1.5 cm high, 1.3–1.7 cm in diam., enclosing 1/3 of nut when mature, outside tomentose with whitish hairs to glabrous, inside villous with erect whitish hairs, wall ca. 2–3 mm thick, bracts arranged in 5–6 rings, margin of rings entire (without scale-like structure); nut ovoid, 2.2 cm high, 1.4 cm in diam., blackish, apex acute, basal scar 0.9 cm in diam., convex, glabrous. Fruits characters were obtained from the fallen materials.

Phenology: Unknown. Fallen fruits were collected in February.

Distribution and habitat: VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park. At present, this species is known only from the type locality.

Additional specimens examined: Vietnam. Lam Dong Province, Lan Tranh, 12°04'08.5"N, 108°21'55.5"E, alt. 1,695 m, 18 June 2015, *N. Nguyen, D. Luong, B. Hoang V3202* (DLU, FU).

Etymology: The specific epithet "*bidoupensis*" is derived from its type locality.

Quercus blaoensis A.Camus, Chênes Atlas 2: 121, pl. 229 (1935–1936), nom. nud.; Les Chênes 1: 293 (1935).

Fig. 2.8

Type: VIETNAM. "Station agricole de Blao, province du haut Donaï", 800 m, 25 Apr. 1933, E. Poilane 22372 (lectotype: P [P00754000!]; isolectotypes: P [P00753999!], K [K000832201, K000832202, K000832203, K000832204, image!], G [G00358072, image!], designated here).

Distribution and habitat: VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve. This species was found in hill evergreen forest at 225 m and 1067 m altitude.

Additional specimens examined: VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve, 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 24 Feb. 2014, *Toyama H., Dang V Son, Tagane S.,*

Fuse K., Yahara T., Nagamasu H., Hop Tran V1366 (FU, VNM, the herbarium of Hon Ba Nature Reserve*)*; Ngoc Linh Nature Reserve, 15°10'05.7"N, 107°45'23.6"E, alt. 1067 m, 11 Feb. 2017, *Tagane S., Nagamasu H., Nguyen N., Hoang B., Hoang S., Yang CJ. V6136* (DLU, FU, the herbarium of Ngoc Linh Nature Reserve).

Note: Camus (1935) described *Quercus blaoensis* based on the specimen *Poilane* 22372 from Vietnam. We examined specimens of *Poilane* 22372 in P ([P00754000], [P00753999]), and the digitized images of the specimens in K ([K000832201, K000832202, K000832203, K000832204]) and G [G00358072]. Among them, only two specimens in P are fertile and only P00754000 represents diagnostic traits of a nut with cupule. Thus, we selected the specimen *Poilane* 22372 deposited in P [P00754000] as lectotype for *Q. blaoensis*.

Quercus cambodiensis Hickel & A.Camus, Bull. Mus. Natl. Hist. Nat. 29: 600 (1923); [P. H. Lecomte et al.] Fl. Indo-Chine 5: 946 (1929). *Quercus auricoma* auct. non A.Camus; Tagane et al., Tree Fl. Bokor National Park: 277 (2017).

Fig. 2.9

Type: CAMBODIA. ‘‘Mont. De Elephant, sol argileux tourbeu’’, 1,000 m, 6 Aug. 1919, *E. Poilane 215* (lectotype: P [P00379257!]; isolectotypes: P [P00379258!], NY [00253790, image!], designated here).

Distribution and habitat: CAMBODIA. Kampot Province, Bokor National Park. *Quercus cambodiensis* is common in moist evergreen forest on the top plateau of Mt. Bokor.

Additional specimens examined: CAMBODIA. Kampot Province, Bokor National Park: 10°37'32.30"N, 104°05'15.84"E, alt. 844 m, 17 Oct. 2012, *Tagane S., Fuse K., Choeung HN. C4302* [fr.] (FU, the herbarium of Forest Administration of Cambodia); 10°37'15.48"N, 104°05'10.71"E, 888 m, 9 Dec. 2011, *Toyama H., Tagane S., Ide T., Phourin C., Nagamasu H., Yahara T. 1834* [fr.] (FU, the herbarium of Forest Administration of Cambodia); 10°38'12.59"N, 104°02'06.37"E , 1014 m, 4 Dec. 2011, *Toyama H., Tagane S., Kajisa T., Sakata K., Nobayashi M., Mihara N., Ide T., Chhang P., Nagamasu H. 1458 & 1541* (FU, the herbarium of Forest Administration of Cambodia); ; 10°37'16.77"N , 104°01'52.32"E , 1043 m, 17 Dec. 2013, *Toyama H., Fuse K., Iwanaga F., Rueangruea S., Suddee S., Kim W., Loth M. 6276* (FU, the herbarium of Forest Administration of Cambodia); 10°38'12.59"N, 104°02'06.37"E, 1000 m, 12 Dec. 2013, *Fuse K., Suddee S., Rueangruea S., Iwanaga F., Loth* M. Fuse K. 6342 (FU, the herbarium of Forest Administration of Cambodia).

Note: Hickel and Camus (1923) described *Quercus cambodiensis* Hickel & A.Camus based on two specimens collected by E. Poilane (*Poilane 215 and Poilane 270*) from Cambodia. Deng et al. (2010) selected *Poilane 215* as lectotype for *Q. cambodiensis* (Deng et al. 2010). However, there are three specimens of *Poilanei 215* in P [P00379257, P00379258] and NY [00253790], among which only one specimen [P00379257] represents the diagnostic traits of nuts and cupules. Thus, we selected specimen [P00379257] as lectotype of *Q. cambodiensis*.

Quercus camusiae Trel. ex Hickel & A.Camus, Fl. Indo-Chine 5: 957 (1929). *Quercus geminata* Hickel & A.Camus, Bull. Mus. Natl. Hist. Nat.: 599 (1923), nom. illegit. *Cyclobalanopsis camusiae* (Trel. ex Hickel & A.Camus) Y.C.Hsu & H.W.Jen, J. Beijing Forest. Univ. 15(4): 44 (1993).

Fig. 2.10

Type: VIETNAM. Annam [Trung Ky]: ‘‘Pres de Nha-trang, massif de Honba,’’ 1,000–1,500 m, 18–20 Sep. 1918, *A. Chevalier 38650* (lectotype: P [P00379252!]; isolectotype: P [P00379253!], designated here).

Distribution and habitat: VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve. *Quercus camusiae* was found on slop of lower montane evergreen forest at 995 m, 1336 m and 1498 m altitude.

Additional specimens examined: VIETNAM. Khanh Hoa Province, Hon Ba Nature Reserve, 12°07'08.64"N, 108°56'51.99"E, alt. 1,498 m, 18 July 2013, *Tagane S., Yahara T., Nagamasu H., Fuse K., Toyama H., Tran H., Son VD. V290* (FU, the herbarium of Hon Ba NR, VNM); 12°07'10.02"N, 108°56'51.71"E, alt. 995 m, 19 July 2013 [young fr.], *Tagane S., Yahara T., Nagamasu H., Fuse K., Toyama H., Tran H., Son VD. V342* (FU, the herbarium of Hon Ba NR, VNM); 12°07'11.42"N, 108°57'25.76"E, alt. 1336 m, 25 Nov. 2014, *Toyama H., Tagane S., Dang VS., Nagamasu H., Naiki A., Tran H., Yang CJ. V2173* (FU, VNM, the herbarium of Hon Ba Nature Reserve).

Note: Quercus camusiae Trel. ex Hickel & A.Camus was described by Hickel & Camus (1929) based on specimen collected by A. Chevalier from Vietnam (“Annam: Pres de Nha-trang, massif de Honba”) to replace for name *Q. geminate* Hickel & Camus (1923) without any collection number. Later, Camus (1938) cited specimen *Chevalier 38650* from Vietnam as type specimen of *Q. camusiae*. We found two specimens *Chevalier 38650* in P comprising [P00379252] and

[P00379253], among which only one specimen [P00379252] represents the diagnostic traits of nuts and cupules. Thus, we selected specimen [P00379252] as lectotype of *Q. camusiae.*

Quercus dilacerata Hickel & A.Camus, [P. H. Lecomte et al.] Fl. Indo-Chine 5: 960 (1929).

Type: VIETNAM. "Tonkin: Km. 8 du col de Lo qui Ho près de Chapa", 1800 m, 29 July 1926, *E. Poilane 12645* (lectotype: P [P00753996!]; isolectotype: P [P00753997!], designated here).

Distribution and habitat: VIETNAM. Lao Cai Province: Lo Qui Ho Pass, Chapa.

Note: In the original publication of *Quercus dilacerata,* Hickel and Camus (1929) cited the specimen collected by E. Poilane from Tonkin, Km. 8 du col de Lo qui Ho près de Chapa, Vietnam without any collection number. We found two specimens of *Quercus* collected by Poilane from Tonkin, Km. 8 du col de Lo qui Ho près de Chapa, Vietnam in P with collector's number *12645* (P [P00753996], [P00753997]). Both specimens are fertile and consistent with the description of Hickel and Camus (1929). Here, we designated the specimen [P00753996] with more nuts as lectotype for *Q. dilacerata.*

Quercus donnaiensis A.Camus, Chênes Atlas 2: 119, pl. 227 (1935–1936), nom. nud.; Les Chênes 1: 190 (1935).

Fig. 2.11

Type: VIETNAM. "Annam: Près de Sapoum, près station agricole de Blao, prov. du Haut Donai", 1000–1100 m, 9 Jan. 1935, *E. Poilanei 23732* (lectotype: P [P00753995!]; isolectotype: P [P00753994!], designated here).

Distribution and habitat: VIETNAM. Lam Dong Province: Lan Tranh wards. In this study, *Q. donnaiensis* was found in lower montane evergreen forest, beside a stream at 1489 m altitude and edge of evergreen forest, at 1695 m altitude.

Additional specimens examined: VIETNAM. Lam Dong Province, Lan Tranh wards, 12°04'08.5"N, 108°21'55.5"E, alt. 1,695 m, 18 June 2015, *N. Nguyen, D. Luong, B. Hoang V3208* (DLU, FU); Lam Dong Province, Bi Doup-Nui Ba National Park, 12°11'19.8"N, 108°40'48.3"E, alt. 1,489 m, 25 Feb. 2016, *Tagane S., Wai J. V4398* (FU, DLU, the herbarium of Bidoup-Nui Ba National Park).

Note: Quercus donnaiensis was described by Camus (1935), based on the specimen *Poilanei 23732* collected from Vietnam and then illustrated by Camus (1935–1936). Two specimens of *Poilanei 23732* deposited in P have acorns and are consistent with the illustration and description

of Camus (1935a, b). Among them, we designated the specimen *Poilane 23732* in P [P00753995] with nuts better representing the diagnostic traits as lectotype of *Q. donnaiensis*.

Quercus honbaensis Binh, Tagane & Yahara**, sp. nov.**

Fig. 2.12

Diagnosis: Quercus honbaensis is distinguished from *Q. langbianensis s. str.* in having shorter petiole of 0.8–1 cm long (vs. 1–1.8 cm long), more secondary veins ((10–)14–16 pairs vs. 10–12 pairs), and obconical cupules (vs. cup-shaped).

Type: VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve, evergreen forest along river, 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 24 Feb. 2014, *Toyama H., Dang V Son., Tagane S., Fuse K., Yahara T., Nagamasu H., Hop Tran V1378* (holotype: KYO!; isotypes: FU!, VNM!).

Description: Tree, 12 m tall. Buds ovoid, ca. 3–4 mm high, ca. 2–3 mm in diam., scales in 4–6 rows, imbricate, ovate-triangular, ca. 1 × 1.5 mm, apex obtuse, margin yellowish brown ciliate, appressed hairy on both surfaces. Young twigs grayish brown, 1–1.2 mm in diam., curly hairy, sometimes sulcate, old twigs grayish brown, glabrous, lenticellate. Leaf blades lanceolate to oblanceolate, (3.6–)11–16.5 × (1.4–)2–5.2 cm, acute at apex, cuneate at base, margin regularly distinctly serrate in the upper 5/6–3/4(–2/3), glabrous on both surfaces; midrib slightly prominent adaxially, prominent abaxially, lateral veins (9–)10–14(–16) pairs, straight and running into the teeth of margin, slightly prominent adaxially, prominent abaxially, at angle of 40–45 degree from midrib, tertiary veins scalariform-reticulate, faintly visible on both surfaces; petioles 0.8–1 cm long, tomentose when young, soon glabrous. Male and female inflorescences not seen. Infructescences axillary, erect, rachis 0.5–1.4 cm long, 1–2 mm in diam., glabrous. Mature fruits 2–3.5 cm high (including cupule), usually 1–(2) per infructescence, sessile; cupules obconical, 1.4–1.6 cm high, 1.5–1.8 cm in diam., enclosing 1/3–1/2 of the nut, wall covered with densely whitish to yellowish brown hairs, bracts arranged in 6–8 rings, margin of the ring undulate; nut obovoid to ellipsoid, 2.3–2.8 cm high, 1.3–1.7 cm in diam., apex obtuse, sparsely hairy except densely appressed hairy around stylopodia and basal scar, stylopodia up to 3 mm long, basal scar 0.7–0.8 cm in diam., convex.

Phenology: Fruiting specimens were collected in February.

Distribution and habitat: VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve. This species is known only from the type locality. A few individuals were found in evergreen forest from 225–617 m elevation.

Additional specimens examined: VIETNAM. Khanh Hoa Province: Hon Ba Nature Reserve, 12°06'33.41"N, 108°59'24.89"E, alt. 367 m, 22 July 2013, *Tagane S., Yahara T., Nagamasu H., Fuse K., Toyama H., Tran H., Dang V.S., V744* (FU, VNM, the herbarium of Hon Ba NR); 12°06'39.77"N, 108°58'59.23"E, alt. 617 m, 22 Feb. 2014, *Toyama H., Dang V.S., Tagane S., Fuse K., Yahara T., Nagamasu H., Tran H., Nguyen V.N., Nguyen Q.C., Do N.T., Ho N.P.H., V1200* (FU, VNM, the herbarium of Hon Ba NR); 12°06'31.2"N, 108°59'14.1"E, alt. 400 m, 13 July 2014, *Tagane S., Kanemitsu H., Dang V.S., Tran H., Hanh N., Loi X.N., Thach N.D., Dinh N., Hieu P.N.H., V1548* (FU, VNM, the herbarium of Hon Ba NR); 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 15 July 2014, *Tagane S., Kanemitsu H., Dang V.S., Tran H., Loi X.N., Thach N.D., Dinh N., Hieu P.N.H., V1662* (FU, VNM, the herbarium of Hon Ba NR).

Etymology: The specific epithet "*honbaensis*" is derived from its type locality, Mt. Hon Ba.

Quercus langbianensis Hickel & A.Camus, Ann. Sci. Nat., Bot. 10, 3: 382 (1921); [P.H. Lecomte et al.] Fl. Indo-Chine 5: 950 (1929).

Fig. 2.13

Type: VIETNAM. "Annam: massif du Lang-Bian, grand Piton Lang-Bian, près du village de Beneur", 1500–2000 m, 15 Feb. 1914, *A.J.B Chevalier 30029* (holotype: P [P00379254!]; isotypes: P [P00379255! P00379256!]).

Distribution and habitat: VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park. At present, *Quercus langbianensis s. str.* is known only from Lang-Bian Mountain of the national park, in lower montane evergreen forest at 1472 m and 1533 m altitude.

Additional specimen examined: VIETNAM. Bidoup-Nui Ba National Park, 12°10'34.04"N, 108°40'28.93"E, alt. 1,472 m, 29 Feb. 2016, *Tagane S.*, *Son VD., Wai J. V4465* (DLU, FU, the herbarium of Bidoup-Nui Ba National Park)*;* ibid. 12°10'34.09"N, 108°41'04"E, alt. 1,533 m, 22 Feb. 2016, *Tagane S.*, *Nagamasu H., Naiki A., Dang V. Son, Nguyen V. Ngoc., Binh T. Hoang V4165, V4166* (DLU, FU, the herbarium of Bidoup-Nui Ba National Park)*;* 12°10'34.7"N, 108°41'08.4"E, alt. 1,533 m, 21 Feb. 2016, *Tagane S.*, *Nagamasu H., Naiki A., Dang V. Son, Nguyen V. Ngoc., Binh T. Hoang V3962* (DLU, FU, the herbarium of Bidoup-Nui Ba National Park).

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Legends

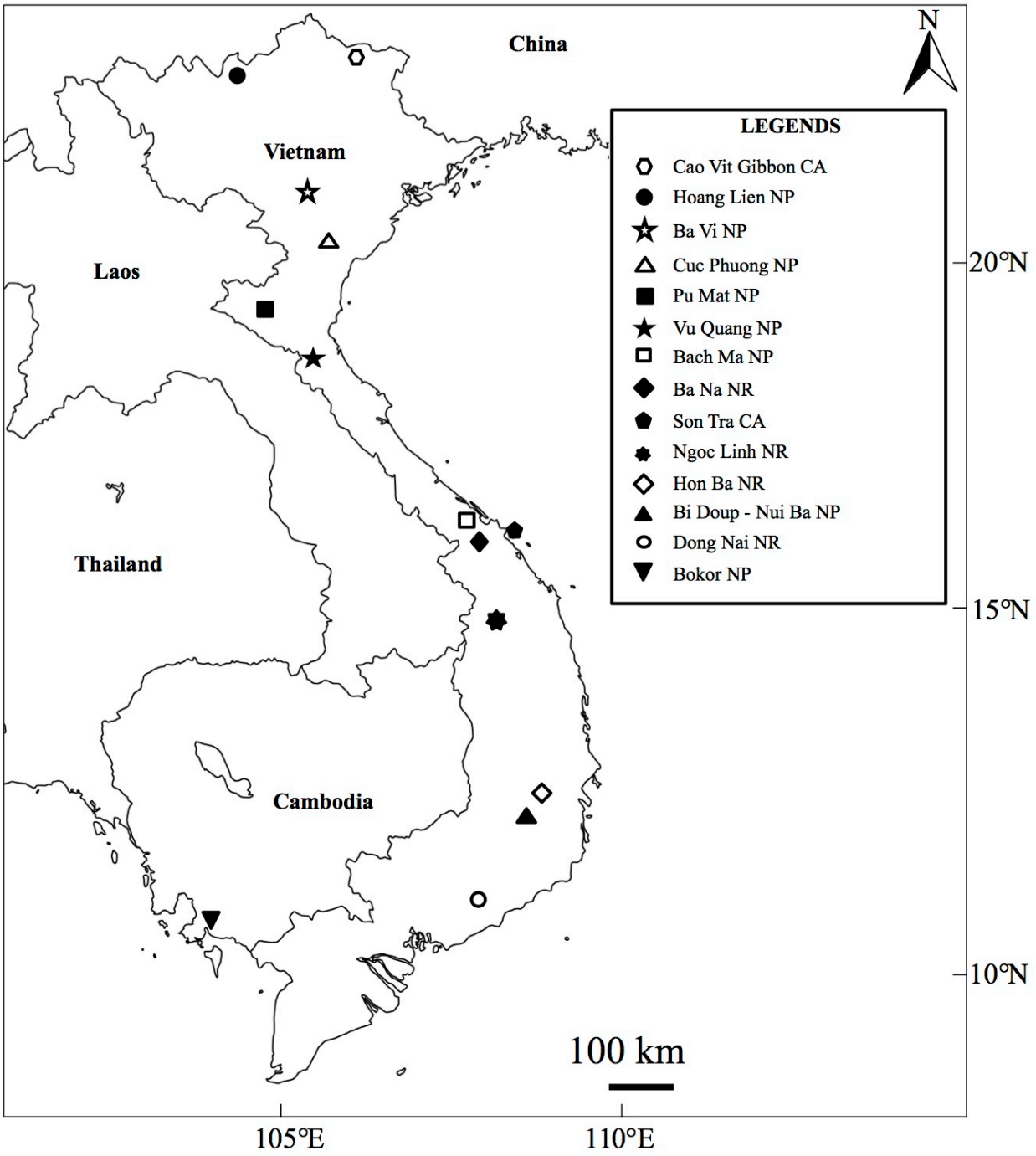


Figure 2.1. Collection sites in Vietnam and Cambodia in this study, including eight national parks, four nature reserves and two conservation areas.

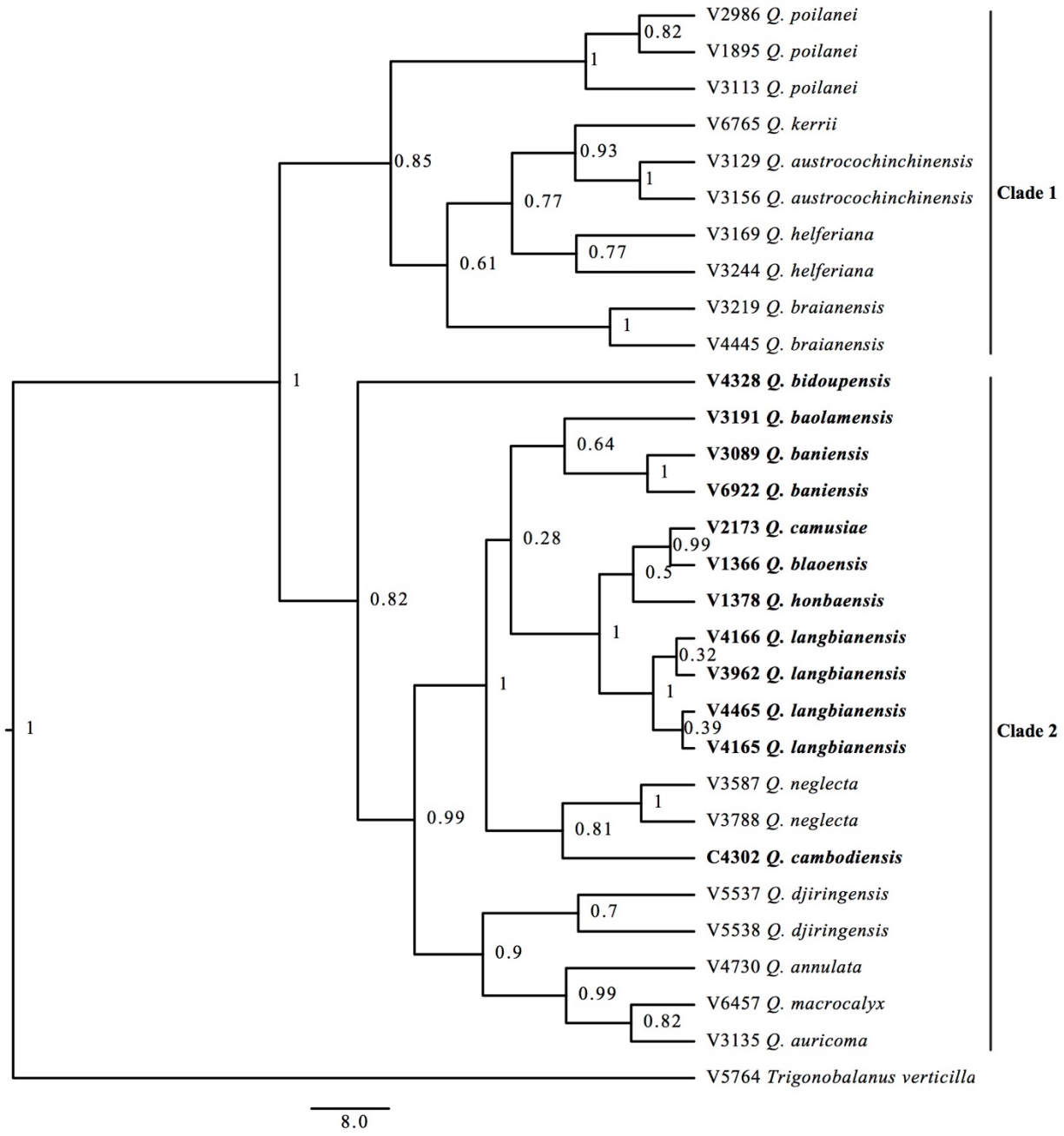


Figure 2.2. Bayesian phylogeny of 29 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on *rbc*L, *mat*K and ITS sequences. Braches are labeled with posterior probabilities.

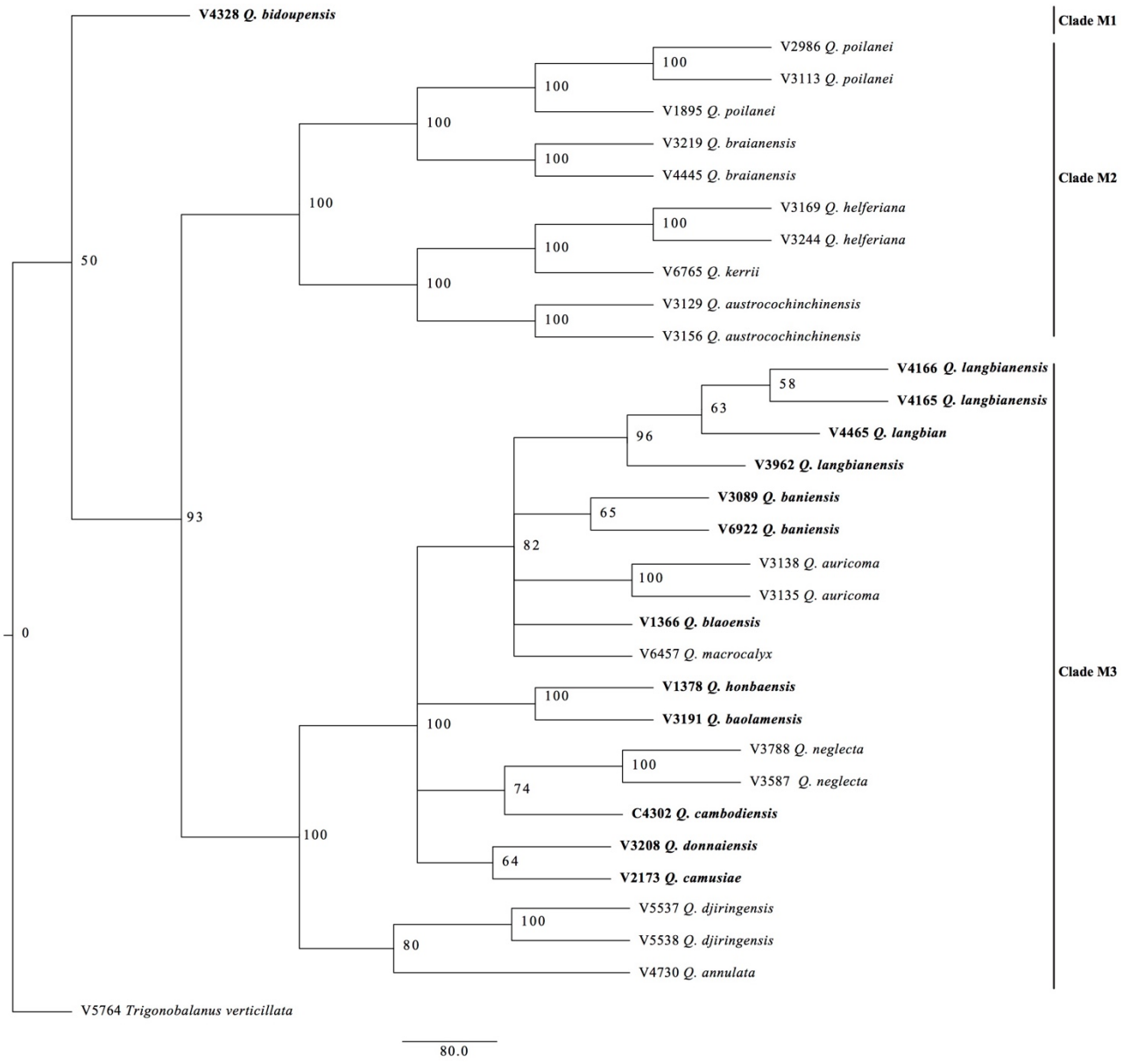


Figure 2.3. NJ tree of 31 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on presence/absence data of 16,809 MIG-seq loci. Branches are labeled with bootstrap supports (% of 1000 replicates).

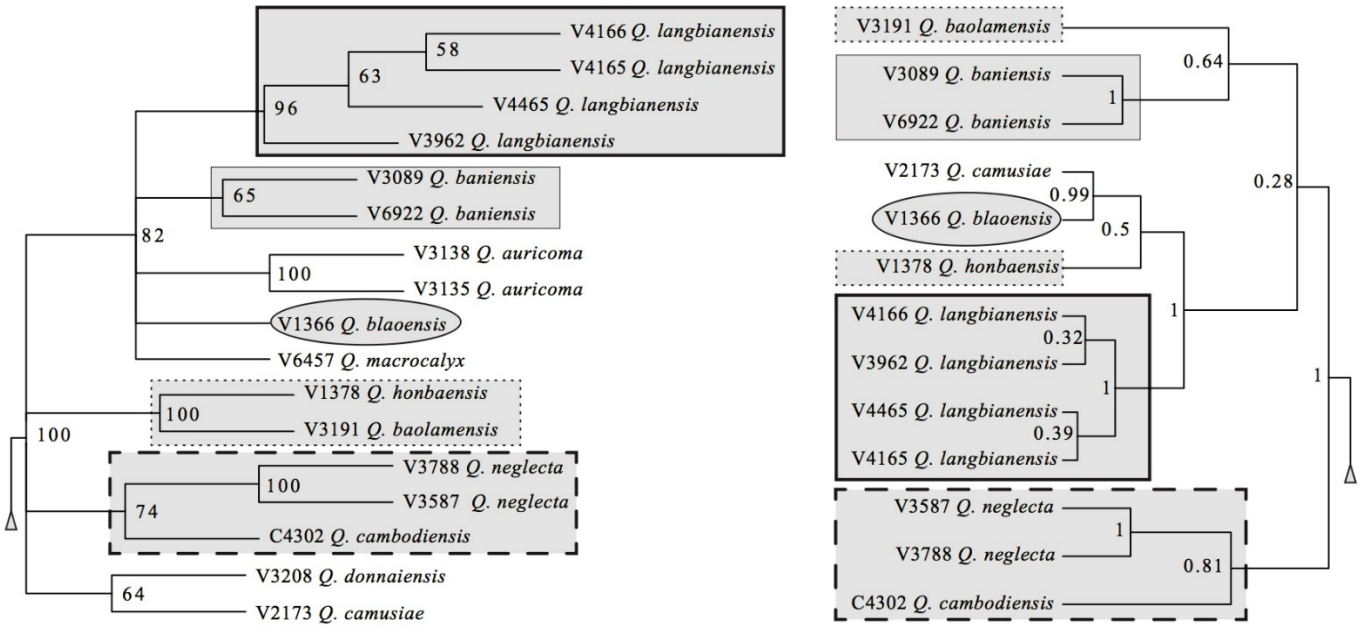


Figure 2.4. Comparison of *Q. langbianensis* complex between NJ tree (left, Clade M3 of Fig. 2.3) and Bayesian tree (right: Clade 2 of Fig. 2.2).

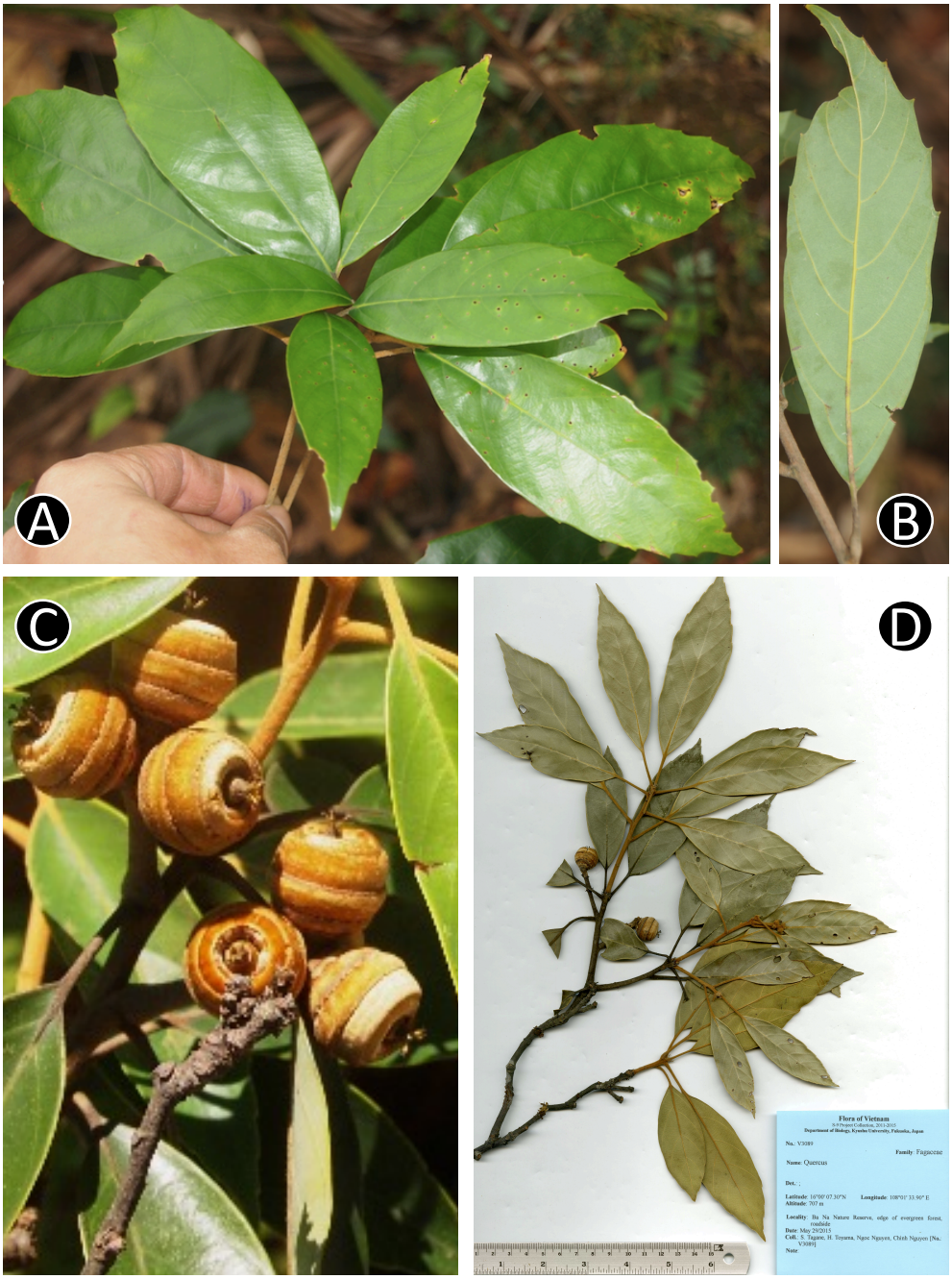


Figure 2.5. *Quercus baniensis* A.Camus **A.** Leafy twig, **B.** Abaxial side of mature leaf, **C.** Infructescence and young fruits, **C.** Dried specimen. Materials: **A & B** from *Hoang T.S. & Tagane S. V6922,* **C & D** from *Tagane et al. V3089.*

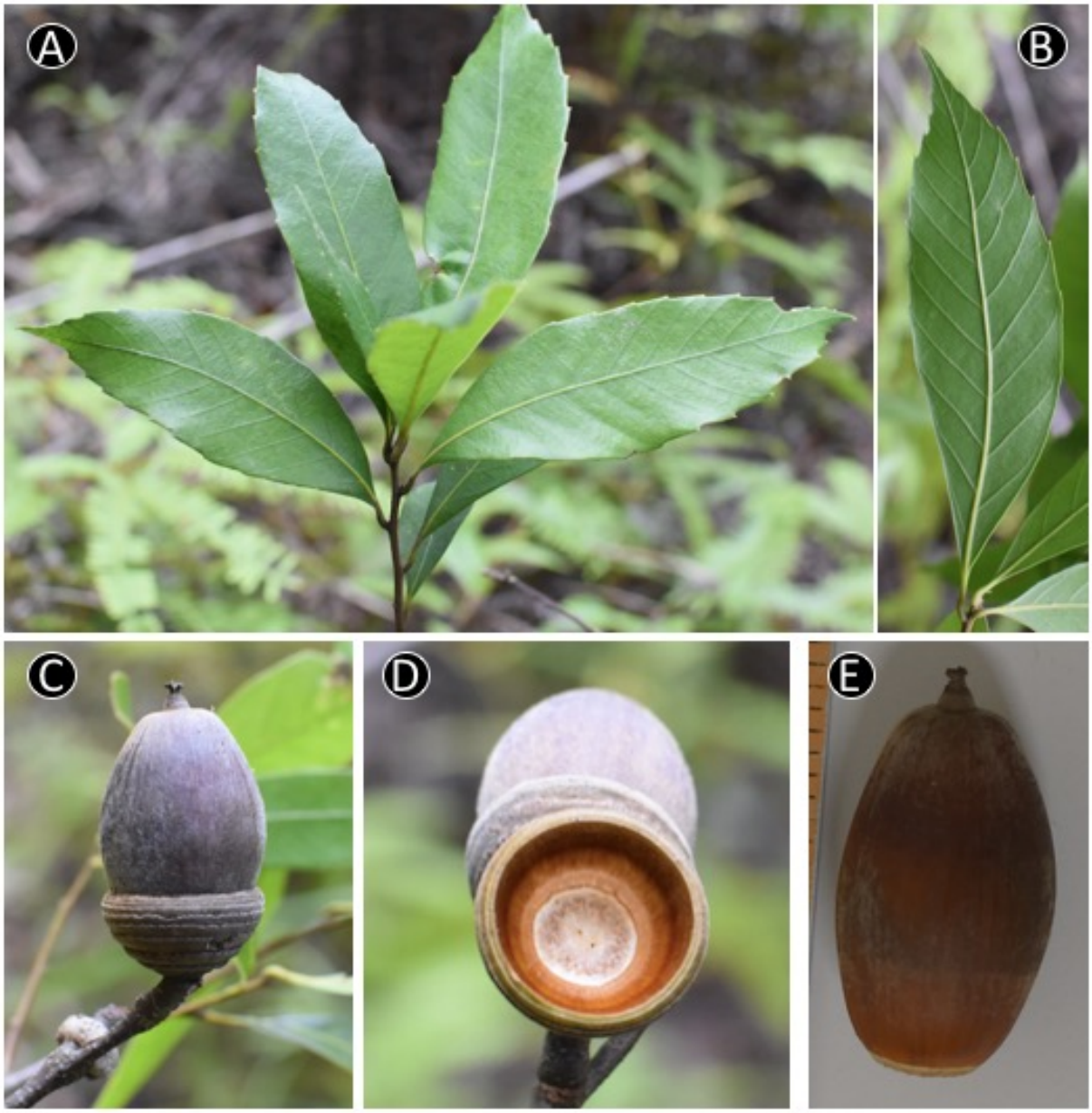


Figure 2.6. *Quercus baolamensis* Binh & Ngoc. **A.** Leafy twig, **B.** Abaxial side of mature leaf, **C.** Mature fruit, **D.** Inside of cupule, **E.** Nut. Materials: **A–E** from *Ngoc et al. V3191*).

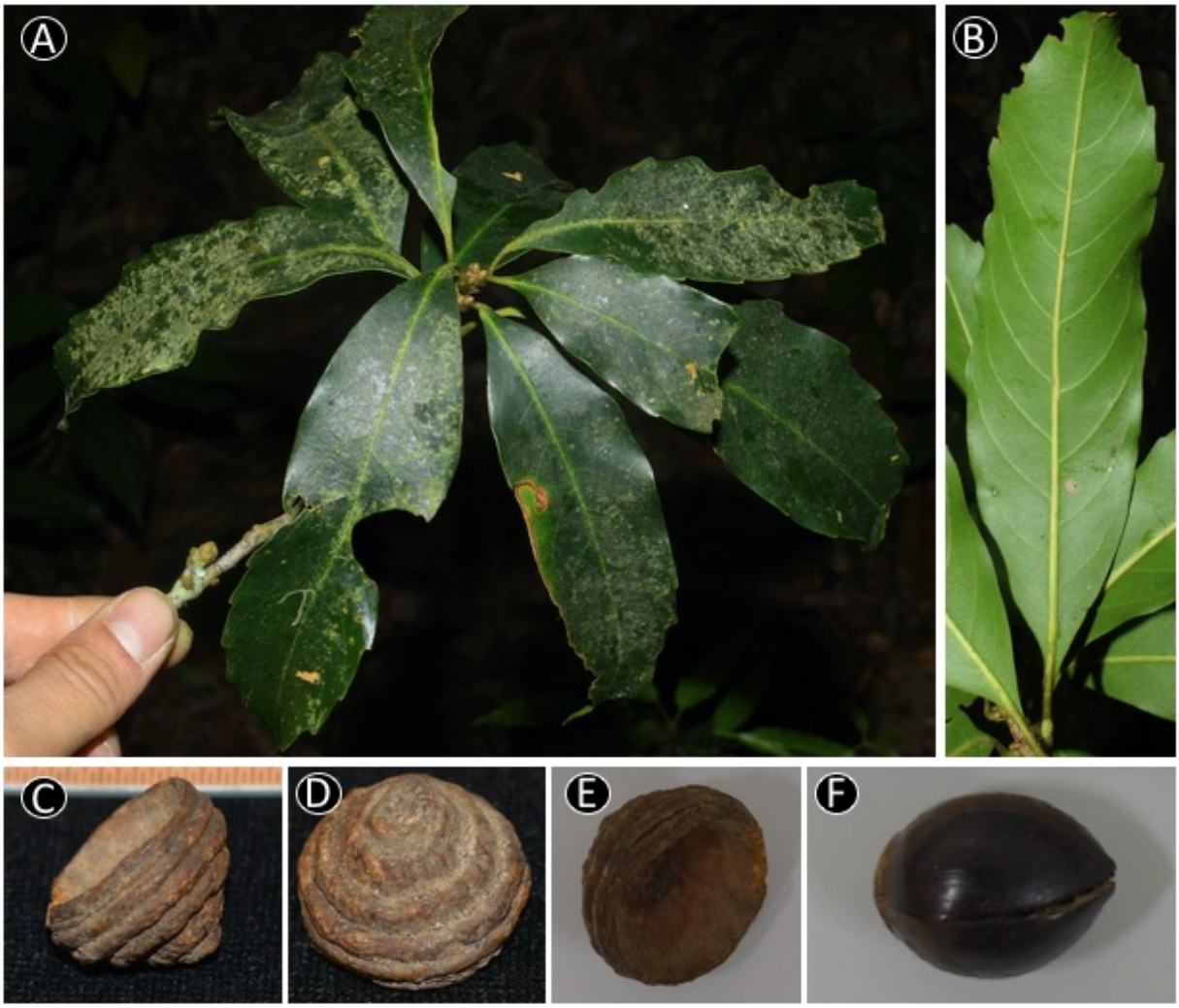


Figure 2.7. *Quercus bidoupensis* Binh & Ngoc. **A.** Leafy twig, **B.** Abaxial side of mature leaf, **C & D.** Side view and base view of the cupule, respectively, **E.** Inside of cupule, **F.** Nut. Materials: **A–F** from *N. Nguyen et al. V4328*).

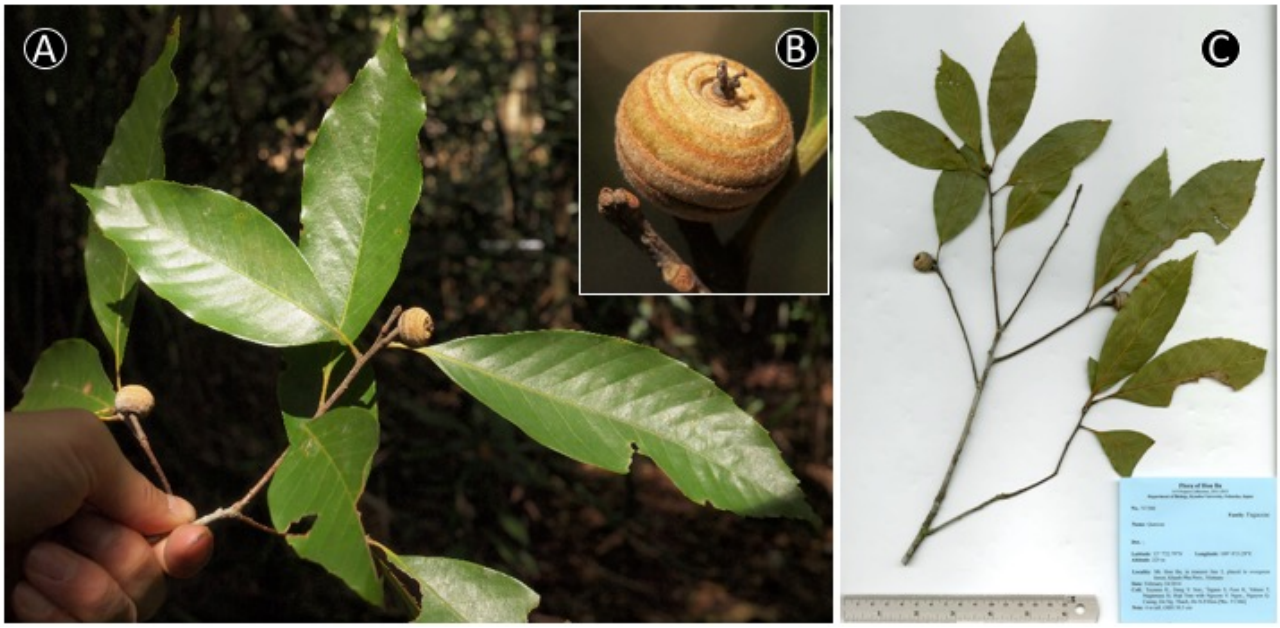


Figure 2.8. *Quercus blaoensis* A.Camus **A.** Branch with fruits, **B.** Young fruit, **C.** Dried specimen Materials: **A–C** from *Toyama et al. V1366*).

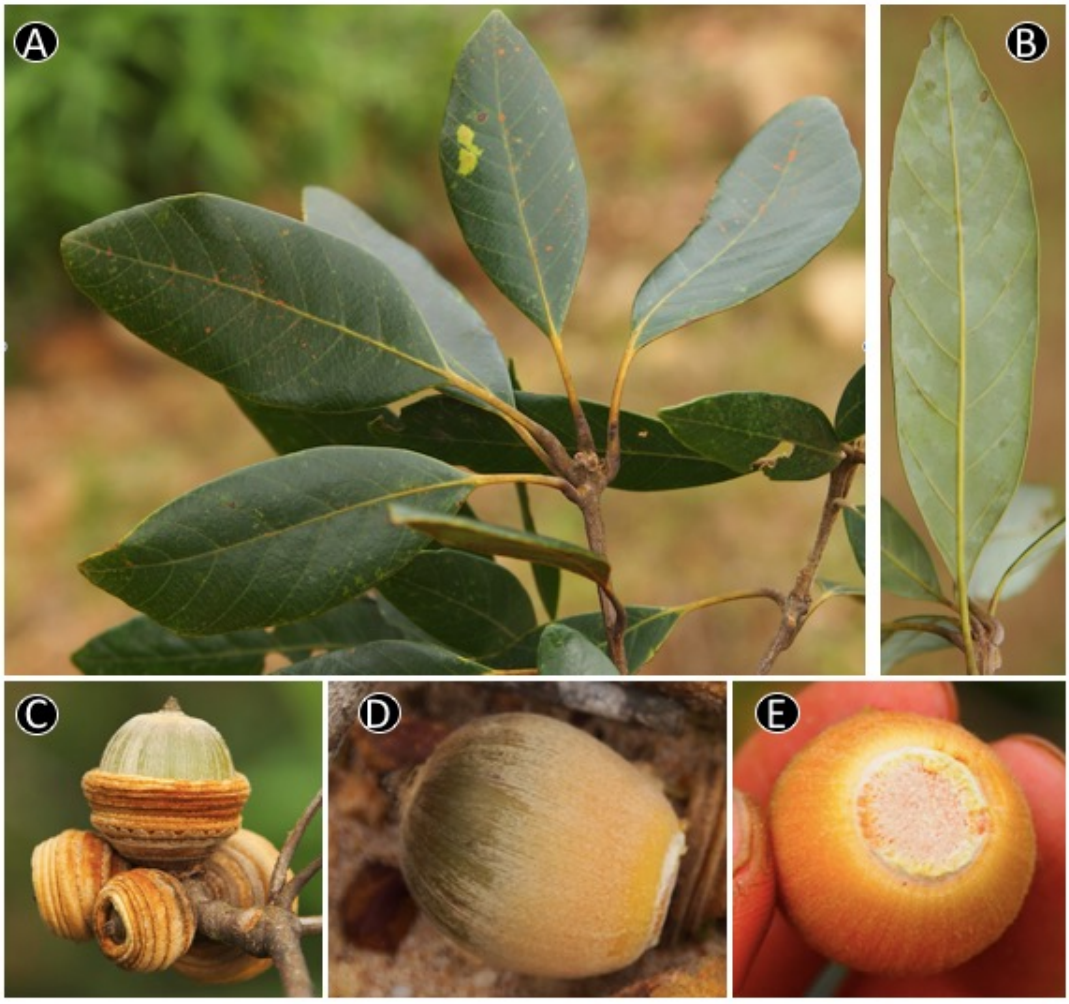


Figure 2.9. *Quercus cambodiensis* Hickel & A.Camus **A.** Leafy twig, **B.** Abaxial side of mature leaf, **C.** Infructescence and fruits, **D.** Nut, **E.** Basal scar of the nut. Materials: **A–E** from *Tagane et al. 4302*).

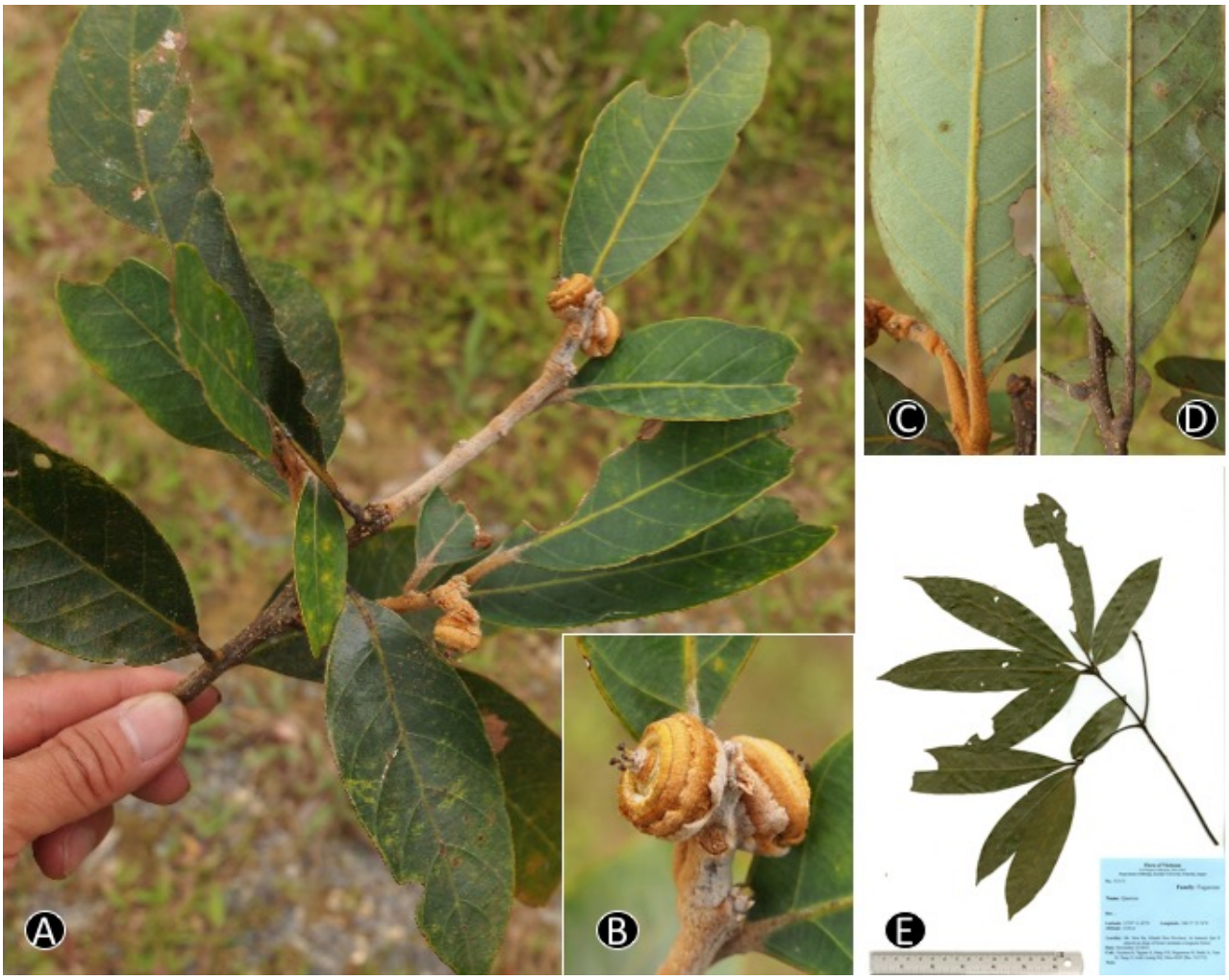


Figure 2.10. *Quercus camusiae* Trel. ex Hickel & A.Camus **A.** Branch with young fruit, **B.** Infructescence and young fruits, **C & D.** Abaxial side of young and mature leaf, **E.** Dried specimen. Materials: **A–D** from *Tagane et al.* V342, **E** from *Toyama et al. V2173*).

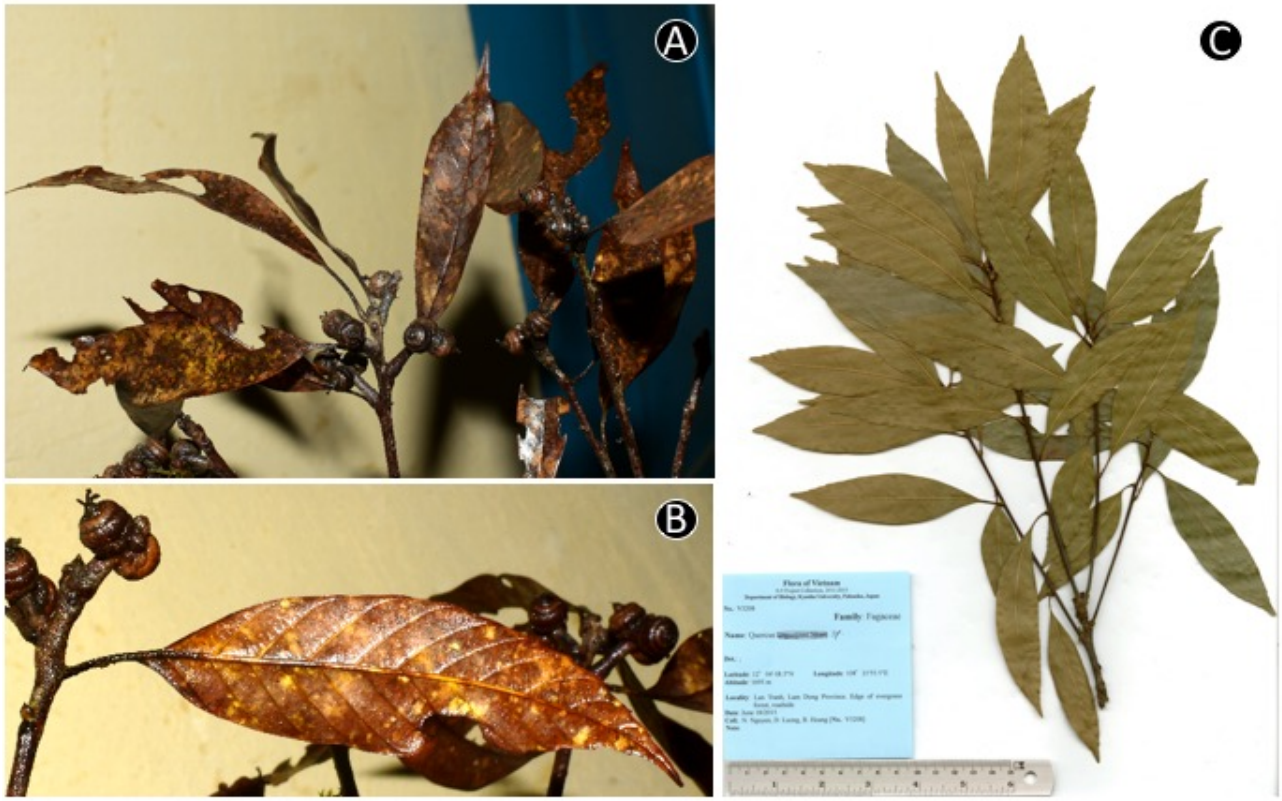


Figure 2.11. *Quercus donnaiensis* A.Camus **A.** Leafy twig, **B.** Infructescence, young fruits and abaxial side of mature leaf, **C.** Dried specimen. Materials: **A & B** from *Tagane S., Wai J. V4398,* **C** from *Ngoc et al. V3208*).

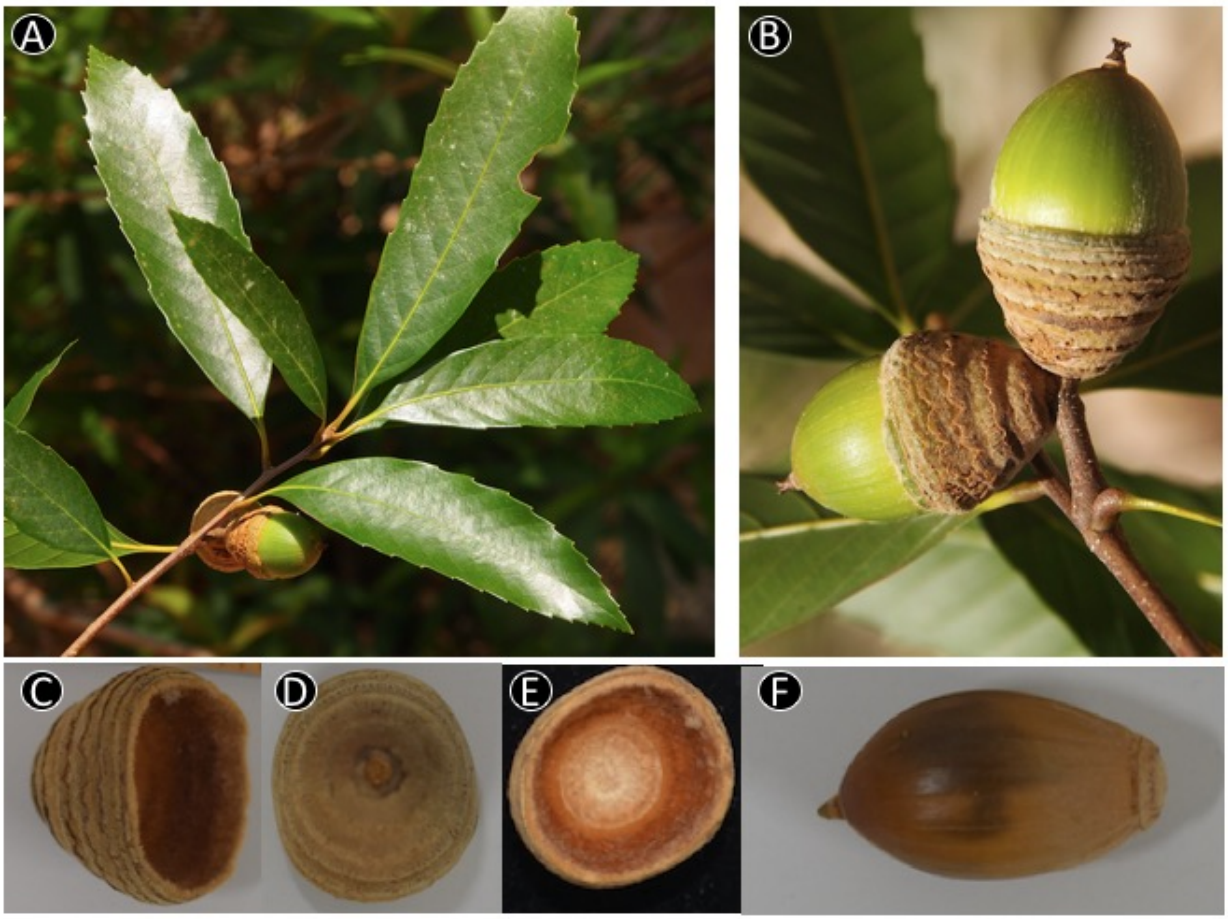


Figure 2.12. *Quercus honbaensis* Binh, Tagane & Yahara **A.** Leafy twig, **B.** Infructescence and mature fruits, **C** & **D.** Side view and base view of the cupule, respectively, **E.** Inside of cupule, **F.** Nut. Materials: **A–F** from *Toyama et al. V1378*).

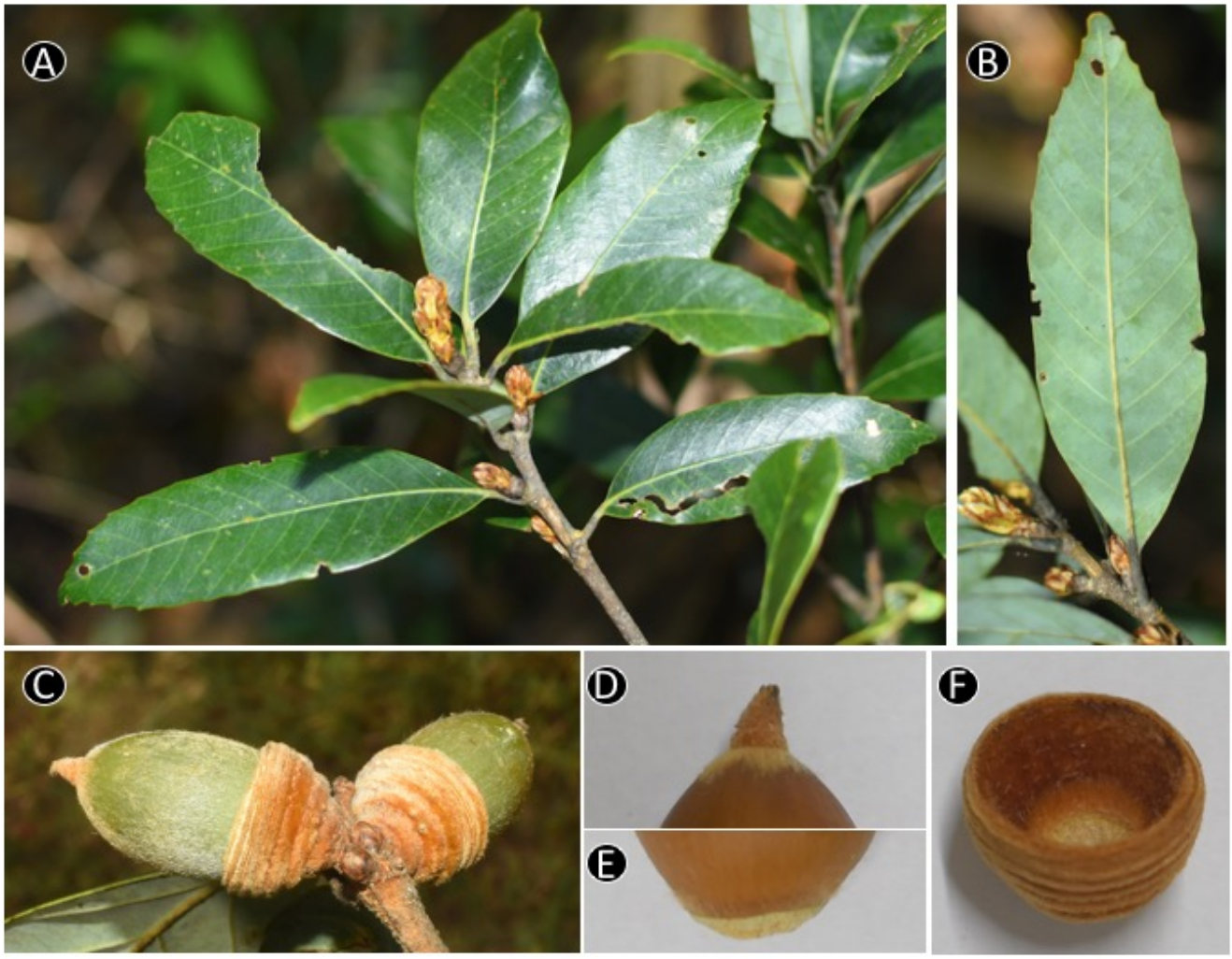


Figure 2.13. *Quercus langbianensis* Hickel & A.Camus **A.** Leafy twig, **B.** Abaxial side of mature leaf, **C.** Infructescence and mature fruits, **D.** Apex of the nut, **E.** Basal scar of the nut, **F.** Inside of cupule. Materials: **A & B** from *Tagane et al. V 4165,* **C–F** from *Tagane et al. V4166*).

Table 2.1. Altitudinal distribution of *Quercus* spp. found in Mt. Hon Ba.

Altitude (m)	*Quercus* trees found in each plot (tree height, girth)
1498	*Q. camusiae* (16 m, 88.5 cm)
1336	*Q. camusiae* (4.5 m, 4.8 cm)
1204	*Q. poilanei* (25m, 86.5 cm; 25 m, 114 cm)
1021	No *Quercus* species
919	No *Quercus* species
617	*Quercus honbaensis* (12 m, 51.4 cm)
400	*Quercus honbaensis* (8 m, 19 cm; 18 m, 88 cm; 5 m, 10 cm)
225	*Quercus honbaensis* (4 m, 6.8 cm), *Q. blaoensis* (14 m, 50.5 cm; 11 m, 15.4 cm)

Table 2.2. Summary statistics of datasets used for phylogenetic inference comprising *rbc*L, *mat*K and ITS sequences.

Regions	*rbc*L	*mat*K	ITS	Combined data
Aligned sequence length	657	834	543	2034
Variable DNA sites	9	35	98	142
Parsimony-informative sites	3	9	44	56

Table 2.3. Morphological comparison of *Quercus langbianensis* complex.

Characters	*Q. bidoupensis*	*Q. camusiae*	*Q. cambodiensis*	*Q. baniensis*	*Q. honbaensis*	*Q. dilacerata*	*Q. blaoensis*	*Q. donnaiensis*	*Q. baolamensis*	*Q. langbianensis*
Young shoot	Almost glabrous	Golden tomentose			Curly hairy	Golden tomentose	Straight hairy	Golden tomentose	Almost glabrous	
Leaf margin	Undulate, distinctly serrate in upper 1/2	Not undulate, almost entire or with a few low teeth in upper 1/4		Not undulate, distinctly serrate in upper 1/3	Not undulate, distinctly serrate in upper 5/6–3/4(–2/3)	Not undulate, distinctly serrate in upper 1/3			Not undulate, distinctly serrate in upper 1/2	Not undulate, distinctly serrate in upper 1/3
Length of petioles	1.3–2 cm	1–1.6 cm	1–2.2 cm	1.2–2(–2.9) cm	0.8–1 cm	1–1.4 cm	0.9–1.8 cm	1–2 cm	0.4–1 cm	1–2 cm
Number of secondary veins	10–13 pairs	8–13 pairs	7–11 pairs		(9–)10–14(–16) pairs	12–14 pairs	8–13 pairs	9–12(–14) pairs	(7–)10–13 pairs	10–12 pairs
Cupule shape	Obconical	Cup-shaped		Obconical		Bowl-shaped	Cup-shaped			
Cupule coverage	Enclosing 1/3 of the nut	Enclosing <1/2 of the nut		Enclosing 2/3 of the nut	Enclosing 1/3–1/2 of the nut	Enclosing 2/3 of the nut		Enclosing 1/2 of the nut	Enclosing 1/3 of the nut	
Cupule bract	5–6 rings	6 rings	7–8 rings	6–8 rings				5–6 rings	6–9 rings	
Cupule bract margin	Entire	Sparsely dissected in the lower rings	Distinctly toothed in two lower rings	Undulate		Distinctly toothed in all rings	Nearly entire, not undulate			
Nut shape	Ovoid	Subglobose		Ovoid	Obovoid to ellipsoid	Subglobose	Ovoid	Subglobose	Ovoid-ellipsoid	Obovoid to ellipsoid
Nut scar	Convex	Flat		Convex				Convex	Flat	Convex
Nut hairiness	Glabrous	Densely hairy		Sparsely hairy			Densely hairy	Sparsely hairy		Densely hairy

Appendix. Voucher information and GenBank accession numbers for samples used in this study (newly sequenced data).

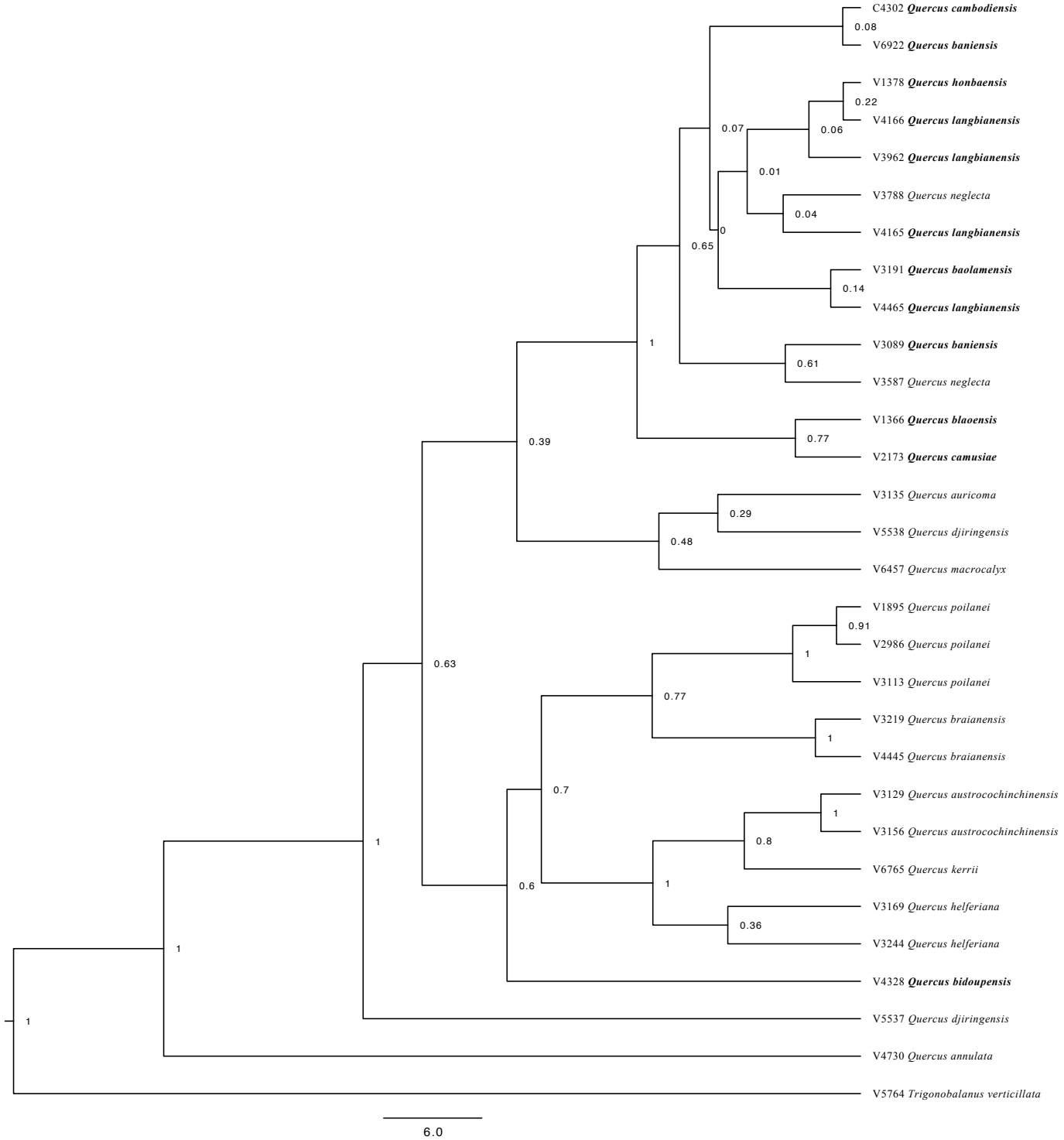
Species	Vouchers	Elevation	GenBank accession no.			MIG-seq
			*rbc*L	*mat*K	ITS	
Quercus annulata	*Tagane et al. V4730*	1850 m	LC318796	LC318516	MF770291	+
Quercus auricoma	*Ngoc et al. V3135*	518 m	LC318778	LC318498	MF770277	+
Quercus auricoma	*Ngoc et al. V3138*	556 m	LC318779	LC318499	-	+
Quercus austrocochinchinensis	*Ngoc et al. V3129*	249 m	LC318777	LC318497	MF770276	+
Quercus austrocochinchinensis	*Ngoc et al. V3156*	340 m	LC318780	LC318500	MF770278	+
Quercus baniensis	*Tagane et al. V3089*	707 m	LC318775	LC318495	MF770274	+
Quercus baniensis	*Hoang & Tagane V6922*	789 m	LC318802	LC318522	MF770296	+
Quercus baolamensis	*Ngoc et al. V3191*	1000 m	LC318782	LC318502	MF770280	+
Quercus bidoupensis	*Ngoc et al. V3202*	1695 m	LC318783	LC318503	-	-
Quercus bidoupensis	*Tagane et al. V4328*	1698 m	LC318793	LC318513	MF770288	+
Quercus blaoensis	*Toyama. et al. V1366*	225 m	LC318768	LC318488	MF770269	+
Quercus blaoensis	*Tagane et al. V6136*	1067 m	LC318799	LC318519	-	+
Quercus braianensis	*Ngoc et al. V3219*	1535 m	LC318785	LC318505	MF770281	+
Quercus braianensis	*Ngoc et al. V4445*	1464 m	LC318794	LC318514	MF770289	+
Quercus cambodiensis	*Tagane et al. 1834*	888 m	-	-	-	-
Quercus cambodiensis	*Tagane et al. 1458*	1014 m	-	-	-	-
Quercus cambodiensis	*Tagane et al. 1541*	1014 m	-	-	-	-
Quercus cambodiensis	*Tagane et al. 6276*	1043 m	-	-	-	-
Quercus cambodiensis	*Tagane et al. 6342*	1000 m	-	-	-	-
Quercus cambodiensis	*Tagane et al. 4302*	844 m	LC318766	LC318445	MF770268	+
Quercus camusiae	*Tagane et al. V290*	1498 m	-	-	-	-
Quercus camusiae	*Tagane et al. V342*	1511 m	LC318787	LC318507	-	-
Quercus camusiae	*Toyama. et al. V2173*	1336 m	LC318773	LC318493	MF770272	+

Appendix. Continued

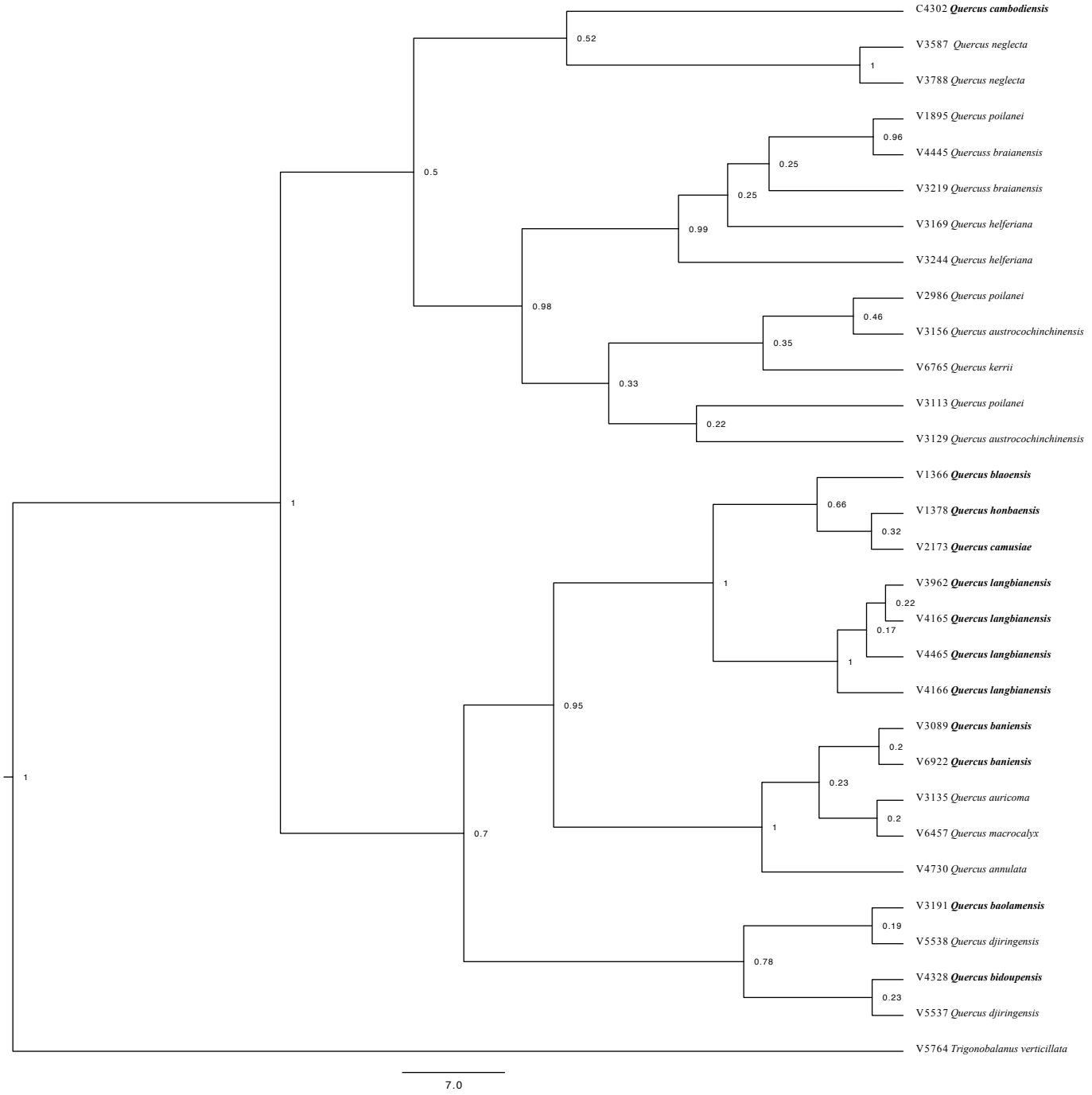
Species	Vouchers	Elevation	GenBank accession no.			MIG-seq
			*rbc*L	*mat*K	ITS	
Quercus donnaiensis	*Ngoc et al. V3208*	1695 m	LC318784	LC318504	-	+
Quercus donnaiensis	*Tagane & Wai 4398*	1489 m	-	-	-	-
Quercus djiringensis	*Dung et al. V5537*	N/A	LC318797	LC318517	MF770292	+
Quercus djiringensis	*Dung et al.* V5538	N/A	LC318798	LC318518	MF770293	+
Quercus helferiana	*Ngoc et al. V3169*	1400 m	LC318781	LC318501	MF770279	+
Quercus helferiana	*Ngoc et al. V3244*	1580 m	LC318786	LC318506	MF770282	+
Quercus honbaensis	*Tagane et al. V744*	367 m	-	-	-	-
Quercus honbaensis	*Toyama. et al. V1200*	617 m	LC318767	LC318446	-	-
Quercus honbaensis	*Tagane. et al. V1548*	400 m	LC318770	LC318490	-	-
Quercus honbaensis	*Tagane. et al. V1662*	225 m	LC318771	LC318491	-	-
Quercus honbaensis	*Toyama. et al. V1378*	225 m	LC318769	LC318489	MF770270	+
Quercus kerrii	*Tagane et al. V6765*	833 m	LC318801	LC318521	MF770295	+
Quercus langbiangensis	*Tagane et al. V3962*	1533 m	LC318790	LC318510	MF770285	+
Quercus langbiangensis	*Tagane et al. V4165*	1533 m	LC318791	LC318511	MF770286	+
Quercus langbiangensis	*Tagane et al. V4166*	1533 m	LC318792	LC318512	MF770287	+
Quercus langbiangensis	*Tagane et al. V4465*	1472 m	LC318795	LC318515	MF770290	+
Quercus macrocalyx	*Tagane et al. V6457*	1376 m	LC318800	LC318520	MF770294	+
Quercus neglecta	*Ngoc et al. V3587*	546 m	LC318788	LC318508	MF770283	+
Quercus neglecta	*Ngoc et al. V3788*	1062 m	LC318789	LC318509	MF770284	+
Quercus poilanei	*Toyama. et al. V1895*	1644 m	LC318772	LC318492	MF770271	+
Quercus poilanei	*Yahara et al. V2986*	1412 m	LC318774	LC318494	MF770273	+
Quercus poilanei	*Tagane et al. V3113*	1310 m	LC318776	LC318496	MF770275	+
Trigonobalanus verticillata (Outgroup)	*Ngoc et al. V5764*	1735 m	LC318965*	LC318549*	MF770380*	+

(+): Using to analyze in this paper; (-): Do not use to analyze in this paper. (*) From GenBank.

Supplement



Supplementary *Figure S1:* Bayesian phylogeny of 29 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on ITS sequences. Braches are labeled with posterior probabilities.



Supplementary *Figure S2:* Bayesian phylogeny of 29 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on concatenated *rbc*L and *mat*K sequences. Braches are labeled with posterior probabilities.

Chapter 3. A new species and two new records of *Quercus* (Fagaceae) from northern Vietnam.

Abstract

A new species, *Quercus xuanlienensis* Binh, Ngoc & Bon, is described from Xuan Lien Nature Reserve, Vietnam. The new species is morphologically similar to *Q. edithiae* Skan, in having 8–11 pairs of secondary veins, bowl-shaped cupules and ellipsoid to cylindrical-ellipsoid and basally convex nuts. It differs in having serrulate leaf margins only at apical 1/5–1/7, almost entire margins of bracts on cupule and much longer nuts. The species is also similar to *Q. fleuryi* Hickel & A.Camus in having leaves glabrous on both surfaces with only an apically serrulate margin but differs in having shorter petioles, cupules enclosing 1/5 of the nut and much longer nuts. In addition, *Q. disciformis* Chun & Tsiang. and *Q. bella* Chun & Tsiang., previously known from China, are newly recorded from Ba Vi National Park, Vietnam.

Introduction

Quercus L. comprises ca. 400–500 species (Nixon 1993, Valencia-A et al. 2016) and has been divided into two subgenera, *Quercus* subgenus *Cyclobalanopsis* (Oerst.) Schneider (ring-cup oaks) characterised by stigma capitate to subcapitate or discoid stigmas, cupule bracts being connate or forming concentric or spiral rings and *Quercus* subgenus *Quercus* (scale-cup oaks) characterised by usually linear ampliate or broadly ampliated stigma, free and imbricate cupule bracts (Nixon 1993, Manos et al. 1999). In Vietnam, according to Ho (2003) and Ban (2005), a total of 43 *Quercus* species were recorded, amongst which 37 species belong to subgenus *Cyclobalanopsis* and six species belong to subgenus *Quercus*. Recently, the following two species were reported and the species of *Quercus* in Vietnam rose to 45 species: *Q. lineata* Blume of subgenus *Cyclobalanopsis* (Li et al. 2016) and *Q. trungkhanhensis* Binh & Ngoc of subgenus *Quercus* (Binh et al. 2018c).

To widen our knowledge on the Fagaceae of Vietnam, field surveys were undertaken by the authors for 13 conservation areas (national parks, nature reserves and conservation area) in Vietnam and a total of 105 *Quercus* samples were collected. Amongst them, during the field surveys in Xuan Lien Nature Reserve and Ba Vi National Park (Fig. 3.1), we discovered three unknown species of the subgenus *Cyclobalanopsis* which were not identical to any of the 38

species of *Cyclobalanopsis* previously recorded from Vietnam (Ho 2003, Ban 2005, Li et al. 2016, Binh et al. 2018c).

Xuan Lien Nature Reserve, Thuong Xuan District, Thanh Hoa Province, North Central Coast of Vietnam, was established in 1999 with a total area of 21,000 ha. Until now, 1,142 species of vascular plants belonging to 620 genera and 180 families have been recorded (Xuan Lien Nature Reserve, 2017). In Fagaceae, 31 species including 17 *Lithocarpus* species (55%), 10 species of *Castanopsis* (32%) and four species of *Quercus* (13%) have been recorded (Xuan Lien Nature Reserve, 2017). Ba Vi National Park, Ha Noi Capital, northern Vietnam was established in 1991 with a total area of 7,377 ha (Fig. 3.1). In this national park, located in the Ba Vi mountain range, 1,201 vascular plant species of 649 genera and 160 families including 19 species of Fagaceae are recorded (Ba Vi National Park, 2008).

In this study, a new species is reported from Xuan Lien Nature Reserve and two species are newly recorded from Ba Vi National Park. A new species is described as *Quercus xuanlienensis* Binh, Ngoc & Bon. The two newly recorded species to the country are *Q. disciformis* Chun & Tsiang. and *Q. bella* Chun & Tsiang.

In addition to the morphological examination, DNA sequences and phylogenetic analyses are helpful for delimiting species (Hebert and Gregory 2005, Dick and Webb 2012). Here, DNA sequences of the three species were compared with those of 20 species in Vietnam to confirm that the three species are divergent and thus distinct from the other species. First, two DNA barcode regions were sequenced, the partial genes for the large subunit ribulose-1,5-bisphosphate carboxylase oxygenase (*rbc*L) and maturase K (*mat*K) as basic DNA barcodes (CBOL Plant Working Group 2009). However, those sequences show limited divergence in the genus *Quercus* and thus multiple gene markers (Hubert et al. 2014, Simeone et al. 2016), RAD-seq (Cavender-Bares et al. 2015, Fitz-Gibbon et al. 2017) and MIG-seq (Suyama and Matsuki 2015, Binh et al. 2018b) have been used to determine phylogenetic relationships in *Quercus*. In particular, Binh et al. (2018b) successfully used MIG-seq to determine the phylogenetic relationship in the *Quercus langbianensis* complex in Vietnam and revise its taxonomy. In this study, the authors compared the MIG-seq of *Q. xualienensis, Q. disciformis* and *Q. bella* with those of 18 *Quercus* species studied by Binh et al. (2018b) and two additional species *Q. platycalyx* Hickel & A.Camus and *Q. quangriensis* Hickel & A.Camus that have cupules similar to *Q. disciformis* and *Q. bella*, to determine their identities and phylogenetic relationships.

Materials and Methods

Morphological observations

The validity of a new species and the identities of newly recorded species were examined based on literature of the genus *Quercus* in Vietnam and its surrounding countries (Camus 1936-1954, Soepadmo 1972, Ho 2003, Huang et al. 1999, Ban 2005, Phengklai 2008, Li et al. 2016, Binh et al. 2018c), authentic specimens including types by visiting the herbaria DLU, HN, FU, P and VNM and using images available on the web of JSTOR Global Plants (https://plants.jstor.org/) and Chinese Virtual Herbarium (http://www.cvh.org.cn/).

DNA extraction

DNA was isolated from silica-gel dried leaf materials following the CTAB method (Doyle & Doyle 1987) with minor modifications, as in Toyama et al. (2016). Before the DNA extraction, dry leaf material was milled by QIAGEN TissueLyser to obtain fine powder and the powder was washed up to five times by 1 ml buffer (0.1 M HEPES, pH 8.0; 2% Mercaptoethanol; 1% PVP; 0.05 Ascorbic acid).

DNA barcoding

DNA regions of the large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase (*rbc*L) and maturase K (*mat*K) were amplified and sequenced following the protocols of Kress *et al.* (2009) and Dunning and Savolainen (2010), respectively.

Next generation DNA sequencing – MIG-seq

DNA products were used from 105 *Quercus* spp. as templates to amplify thousands of short sequences (loci) from a wide variety of genomes using primers designed for "multiplexed ISSR genotyping by sequencing" (MIG-seq, Suyama and Matsuki 2015). Then presence/absence of each locus in each sample was used for phylogenetic tree reconstruction regardless of whether it has SNP or not. According to the MIG-seq protocol of Suyama and Matsuki (2015) with minor modifications as in Binh et al. (2018b), the 1st PCR, multiple non-repetitive regions from various inter-simple-sequence repeat (ISSR) are amplified from genomic DNA by multiplexed PCR with tailed ISSR primers. The 2nd PCR step was performed based on products from the 50 times dilution for each 1st PCR product with deionised water. Then, 3 µl of each 2nd PCR product was

pooled as a single mixture library and purified. Subsequently, the Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA) was used to selected fragments in the size range 350–800 bp. A SYBR green quantitative PCR assay (Library Quantification Kit; Clontech Laboratories, Mountain View, CA, USA) was used to measure the concentration of the size-selected library with approximately 10 pM of libraries. Finally, 10 pM of libraries were used for sequencing on an Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 cycle, Illumina).

Phylogenetic analyses

In MIG-seq, raw data from 105 samples were pretreated and quality control completed following Suyama and Matsuki (2015) as described in Binh et al. (2018b). Subsequently, a list of loci obtained was used for the next steps. This list of loci was detected at least in one individual (1/105=0.01) with the following settings: all samples belong to the same population and threshold frequency of haplotype count in a population (r) = 0.001, a threshold one-order higher than 0.01. Presence/absence (1/0) data of loci were used to compute a distance matrix, construct a neighbour-joining (NJ) tree and the reliability of the tree topology was examined by bootstrapping with 1000 replicates using PHYLIP ver. 3.695 (Shimada and Nishida 2017) as follows; 1000 times re-sampling with Seqboot, distance computation with Restdist, tree construction with NJ and consensus tree construction with Censense. In addition, FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualise the resulting tree. A phylogenetic tree for 105 samples including 43 *Quercus* species amongst 44 species previously recorded in Vietnam (data not shown) was constructed and subsequently reduced to 28 samples by focusing on the clades containing *Q. xuanlienensis, Q. disciformis, Q. bella* and the additional 20 *Quercus* species following Binh et al. (2018b). A total of 19,916 loci were used for the final phylogenetic tree.

Results

Morphological comparison of a new species and two newly recorded species with similar species

The unknown species (*Quercus xuanlienensis*) collected from Xuan Lien Nature Reserve was not morphologically assignable to any of the species recognised in Vietnam and its

surrounding countries. According to Flora of China (Huang et al. 1999) and Illustrated Flora of Vietnam (Ho 2003), *Q. xuanlienensis* is most similar to *Q. edithiae* in leaf size (7–15 × 3–5.8 cm), leaf base (cuneate), petiole length (1.5–2.8 cm long), number of secondary veins (8–11 pairs), cupule shape (bowl-shaped) and nut shape (ellipsoid to cylindrical-ellipsoid). However, *Q. xuanlienensis* is distinct from *Q. edithiae* in having a leaf margin serrated only along its upper 1/5–1/7 (vs. upper 2/3), entire margin of cupule bracts (vs. denticulate except basal 2 or 3 rings) and longer nut (5–6 cm long vs. 4–4.5 cm long) (Table 3.1). *Quercus xuanlienensis* is also morphologically similar to *Q. fleuryi* Hickel & A.Camus in leaf shape and texture, leaf margin serrulate only at apical 1/5–1/7, entire margin of cupule bracts, basally convex nuts, but *Q. fleuryi* (type: *Fleury 37831*, P [P00753925, P00753926]) showed much larger leaves (14–22 × 5–9 cm) than *Q. xuanlienensis* ((6–)8–11 × 3–4.5 cm)). In addition, *Q. xuanlienensis* is distinct from *Q. fleuryi* in having an ellipsoid bud (vs. ovate), shorter petiole (1.5–2 cm long vs. 2.5–4 cm long), smaller and bowl-shaped cupule, (1.3–1.7 cm high, 1.9–2.1 cm in diam. vs. campanulate to cylindrical, 3.6–3.7 cm high, 3.5 cm in diam.), fewer cupule bracts (7–8 rings vs. 10–13 rings), cupules covering 1/4 to 1/3 of a nut (vs. 2/3) and ellipsoid to cylindrical-ellipsoid (vs. ovoid to cylindrical-ellipsoid) and longer nuts (5–6 cm high, 2–2.3 cm in diam. vs. 3–4.5 cm high, 2–3 cm in diam.) (Table 3.1).

According to the key and descriptions in the Flora of China (Huang et al. 1999), the other two unknown taxa from Ba Vi National Park were identified as *Q. disciformis* and *Q. bella*. Excluding slightly thinner leaves and lower teeth, one species is identical with *Q. disciformis* in the following diagnostic characters: leaf blade oblong to obovate-elliptic (6–13 × 2.5–4 cm), margin serrate in the upper 2/3, glabrous on both surfaces when mature; lateral veins 11–13 pairs; petiole ca. 2 cm long; cupule discoid, rim flat when ripe, 3–4 cm in diam., covering base of the nut, scales arranged in 8–10 rings, margin of rings denticulate except apical 2 or 3 entire; nuts oblate 1.5–2 cm high, 2 cm in diam., apex flattened densely appressed hairy. Another species was identified as *Q. bella* having the following characteristics: leaf blade oblong-elliptic to lanceolate (8–15 cm × 2–3.5 cm), base slightly oblique, margin serrate in the upper 1/2; lateral veins 12 pairs of lateral veins; petiole 1–2 cm long; cupule discoid (ca. 0.5 cm × 2.5–3 cm), covering base of the nut, scales arranged in 6–8 rings, margin of rings irregular denticulate; nut oblate nut 1.5–2 cm high and 2.2–3 cm in diam.

DNA barcoding and MIG-seq

The *rbc*L and *mat*K sequences of *Q. xuanlienensis* showed 100% (627/627 bp) and 99% (907/910 bp) homologies with *Q. donnaiensis* and *Q. austrocochinchinensis*, respectively. The *rbc*L and *mat*K sequences of *Q. disciformis* and *Q. bella* showed that 100% (696/696 bp) and 100% (833/833 bp) homologies with each other, respectively.

A phylogenetic tree, inferred using MIG-seq, showed that *Q. xuanlienensis, Q. disciformis* and *Q. bella* are not identical with any of the 20 species from Vietnam. The neighbour-joining (NJ) tree based on MIG-seq data for 28 sample of *Quercus* recognised two major clades using *Trigonobalanus* as an outgroup (Fig. 3.2). Clade 1 with 82% bootstrap value consists of three species of subgenus *Quercus* (*Q. lanata, Q. setulosa* and *Q. trungkhanhensis*) and Clade 2 with 99% bootstrap value consists of 20 species of subgenus *Cyclobalanopsis* including *Q. bella, Q. disciformis* and *Q. xuanlienensis*. These three species were clustered with *Q. quangtriensis, Q. neglecta* and *Q. platycalyx* and a clade of those six species was strongly supported (74% bootstrap value). Amongst the six species, *Q. xuanlienensis* was separated from the other five species forming a clade with a 74% bootstrap value. Four samples of *Q. disciformis* and three samples of *Q. bella* formed two distinct clades, each supported by 100% bootstrap value. *Quercus disciformis* was sister to *Q. bella* and the clade of those two species had an 84% bootstrap value.

Discussions

The results of the NJ tree, based on MIG-Seq data, showed that *Q. disciformis* is sister to but well differentiated from *Q. bella*. These two species were collected in Ba Vi National Park where they co-occur with similar in leaf and nut morphologies, but differ in the coverage of the cupule (Fig. 3.4, less than 1/8 in *Q. disciformis* vs. Fig. 3.5, basal 1/8 to 1/4 in *Q. bella*). According to the Flora of China (Huang et al. 1999), *Q. bella* is recorded from Guangdong, Guangxi and Hainan provinces, whereas *Q. disciformis* is distributed in SW Guangdong, Guangxi, Guizhou, Hainan and Hunan provinces. Ba Vi National Park is located in northern Vietnam, neighbouring Guangxi province and therefore the occurrences of *Q. disciformis* and *Q. bella* there are understandable.

The two species are similar to *Q. platycalyx* and *Q. quangtriensis* in having oblong to oblong-elliptic leaves, usually serrate along leaf margins in the upper 1/2 to 2/3, glabrous on both surfaces when mature, and cupules covering less than 1/3 of the nut and oblate to ovoid nuts

(Huang et al. 1999, Phengklai 2008, Ho 2003). The MIG-seq tree showed that those four species are related; the monophyly of a clade including the four species and *Q. neglecta* was supported by a 77% bootstrap value, although the support for the monophyly of the four species is weaker. The affinity of the four species and *Q. neglecta* was unexpected because *Q. neglecta* is an easily distinguished species having linear leaves and small nuts (Huang et al. 1999, Ho 2003).

The MIG-seq tree showed that *Q. xuanlienensis* is related to the above four species and *Q. neglecta* that is morphologically distinct from the other *Quercus* species. From the four species (*Q. disciformis, Q. bella, Q. quangtriensis* and *Q. platycalyx*), *Q. xualienensis* is clearly distinguished by the leaf margin (leaf margin serrulate only at apical 1/5–1/7 in *Q. xualienensis* vs. serrate in upper 1/2 to 2/3 in the four species) and nut shape (ellipsoid to cylindrical-ellipsoid vs. oblate to ovoid). Thus, *Q. xuanlienensis* is separated as a distinct species from them.

Whereas *Q. edithiae* is morphologically most similar to *Q. xuanlienensis*, the type specimens of *Q. edithiae* collected from Hong Kong (type: *Ford 623*, K [K000832101, K000832102]) are morphologically distinct from *Q. xuanlienensis* in having distinct serrations, denticulate cupule bracts and smaller nuts and the description of *Q. edithiae* in Flora of China (Huang et al. 1999) agrees with the type specimen. The morphological differences between *Q. edithiae* and *Q. xuanlienensis* are as distinct as those between related species of *Quercus* in Vietnam and its surrounding countries. Huang et al. (1999) recorded *Q. edithiae* in Guangdong, Guangxi, Hainan and Vietnam, but no specimen could be found of *Q. edithiae* collected from Vietnam in any herbarium in Vietnam or on the Chinese Virtual Herbarium website (http://www.cvh.org.cn/). Further studies are needed to confirm the occurrence of *Q. edithiae* itself in Vietnam.

The MIG-seq tree (Fig. 3.2) was very helpful in deriving the conclusions contained in this paper. As *Q. disciformis* and *Q. bella* are morphologically similar and were collected from the same locality, it was difficult to ascertain whether these are in fact two distinct species and not variants of a single species without the support of the MIG-seq data. Also, the separation of *Q. xuanlienensis* from the other species in Fig. 3.2 supported the conclusion that it is a new species. The authors also obtained sequences data of *rbc*L and *mat*K but the informative content of those sequences was too low to resolve the relationships amongst such closely related species of *Quercus*. Difficulties were faced in determining the sequences of ITS for *Q. disciformis, Q. bella* and *Q. xuanlienensis*, most likely due to the low quality of the authors' samples. MIG-seq is

applicable to low quality samples and provides finer resolution of the relationship amongst closely related species (Suyama and Matsuki 2015, Binh et al. 2018b). Further studies using MIG-seq would be fruitful to elucidate the diversity of *Quercus* in Vietnam, a centre of oak species richness in SE Asia.

Taxonomic treatments

Quercus xuanlienensis Binh, Ngoc & Bon, **sp. nov.**

Fig. 3.3

Diagnosis. Quercus xuanlienensis is morphologically similar to *Q. edithiae* of China and Vietnam in leaf size (7–15 × 3–5.8 cm), cuneate leaf base, petiole length (1.5–2.8 cm long), number of secondary veins (8–11 pairs), bowl-shaped cupule, ellipsoid to cylindrical-ellipsoid nut and basally convex nut but differs in leaf margin serrulate only at apical 1/5–1/7 (vs. serrate in the upper 2/3), entire bracts of cupule (vs. almost denticulate except basal 2 or 3 rings which is entire), cupule enclosing 1/5 of the nut (vs. enclosing 1/4-1/3 of the nut) and longer nut (5–6 cm long vs. 4–4.5 cm long).

Type: VIETNAM. Thanh Hoa Province, Thuong Xuan District, Xuan Lien Nature Reserve, in evergreen forest around waterfall, alt. 810 m, 19°52'46.7"N, 105°11'34.4"E, 6 Mar. 2017, *Binh HT, Ngoc NV, Bon TN V6967* (holotype KYO!; isotypes DLU!, FU!, P!, VNM!).

Description. Tree, ca. 18 m tall. Buds ellipsoid, ca. 9 mm long, ca. 4 mm in diam., scales imbricate, in 4–5 rows, ovate-triangular, ca. 3 × 2.5 mm, apex obtuse, margin ciliate, appressed whitish to yellowish brown hairy on both surfaces. Twigs glabrous when old, lenticellate. Stipules linear-lanceolate, 10–14 mm long, densely appressed hairy, glabrescent outside, glabrous inside. Leaves alternate; blade leathery, oblong-elliptic or obovate, (6–)8–11(–15) × 3–4.5(–5) cm, apex acuminate, acumen up to 0.6 cm long, base cuneate, margin recurved, serrulate in the upper 1/5–1/7, pale brown on the upper surface, yellowish brown to reddish brown on the lower surface when dry, glabrous on both surfaces; midribs ±flat on upper surface, prominent and distinct on lower surface, lateral veins 8–11 pairs, prominent on lower surface, at an angle of 40–45 degrees from midrib, straight and running into the margin, tertiary veins scalariform, indistinct on upper surface, prominent and distinct on lower surface; petioles 1–2 cm long, glabrous. Male and female inflorescences not seen. Infructescences axillary or terminal, erect, rachis 8–10 mm long, 4–5 mm

in diam., glabrous, brownish red when fresh, blackish when dried. Fruits 6–6.5 cm high (including cupule), solitary or twin, sessile; cupules bowl–shaped, 1.3–1.7 cm high, 1.9–2.1 cm in diam., enclosing ca. 1/5 of the nut when mature, outside whitish to yellowish brown tomentose to glabrous, inside densely appressed yellowish brown hairy, wall ca. 1–2 mm thick, comprising of bracts, bracts arranged in 7–8 rings, margin of rings entire; nuts ellipsoid to cylindrical-ellipsoid, 5–6 cm high, 2–2.3 cm in diam., apex acute, densely appressed yellowish brown hairy around stylopodia, with stylopodia up to 4 mm long, basal scar 9–10 mm in diam., convex, to 3 mm high, glabrous.

Distribution. Vietnam. Thanh Hoa Province, Thuong Xuan District, Xuan Lien Nature Reserve.

Ecology in Vietnam. At present, only one individual was found in evergreen forest, at 810 m altitude.

Etymology. The specific epithet is derived from the district name of the type locality, Xuan Lien Nature Reserve, Thuong Xuan District, Thanh Hoa Province, North Central Coast of Vietnam.

Phenology. Fruiting specimens were collected in March.

GenBank accession no. Binh et al. V6967: LC331257 (*rbc*L), LC331254 (*mat*K).

Preliminary conservation status. Quercus xuanlienensis is known for only one individual inside the protected area of Xuan Lien Nature Reserve. According to the criterion D of the IUCN Red List criteria (IUCN 2012), this species is qualified as Critically Endangered (CR).

Quercus disciformis Chun & Tsiang., J. Arnold Arbor. 28: 324 (1947).

Cyclobalanopsis disciformis (Chun & Tsiang) Y.C. Hsu & H.W. Jen, Acta Bot. Yunnan. 1:148 (1979).

Type: CHINA. "Hsin-I Hsien, Ling-Tung Pao, Chung-Tung", 3 Aug. 1931, *C. Wang 31087* (holotype-IBK [catalogue no. IBK00081941, image!], isotype-IBSC [catalogue no. 0117316, image!]).

Fig. 3.4

Specimens examined in Vietnam. Ha Noi, Ba Vi District, Ba Vi National Park, in evergreen forest: alt. 737 m, 21°04'33.88"N, 105°22'03"E, 12 Sept. 2016, *Binh et al. V 6052, V6053, V6058* [fr.] (FU); alt. 1172 m, 21°03'34.1"N, 105°21'54.1"E, 11 Sep. 2016, *Binh et al. V6040* [fr.] (FU).

Distribution. China (Guangdong, Guangxi, Guizhou, Hainan, Hunan) and Vietnam (Ba Vi National Park).

Ecology in Vietnam. In the field survey, only three individuals were found at an altitude of 737 m and one at 1172 m; in evergreen forest.

Phenology. Flowering from March to April, fruiting from August to September in China (Huang 1999). Fruiting in September in Vietnam.

GenBank accession no. Binh et al. V6058: LC331258 (*rbc*L), LC331255 (*mat*K).

Preliminary conservation status. Quercus disciformis is widely distributed from Guizhou to Guangdong and Hainan in China and not recorded as a threatened species in IUCN (2017). The Vietnamese population in Ba Vi National Park extends its distribution range, representing the south-western limit. Given the situation, the population in Vietnam is locally important but the category Least Concern (LC) (IUCN 2012, Ban et al. 2007) would be appropriate for this species.

Quercus bella Chun & Tsiang., J. Arnold Arbor. 28: 326 (1947).

Cyclobalanopsis bella (Chun & Tsiang) Chun ex Y.C. Hsu & H.W. Jen., J. Beijing Forest. Univ. 15(4): 45 (1993).

Type: CHINA. "Fang-Cheng Hsien, Shi-Wan-Ta Shan", alt. 200–250 m, in sparsely wooded ravine along stream on moist sites, 24 Mar. 1944, *S.H. Chun 4772* (IBSC [catalogue no. 0039624, image!]).

Fig. 3.5

Specimens examined in Vietnam. Ha Noi, Ba Vi District, Ba Vi National Park, in evergreen forest: alt. 600 m, 21°04'40.6"N, 105°22'17.2"E, 11 Sep. 2016, *Binh et al. V 6044, V6038* [fr.] (FU); alt. 703m, 21°04'59.6"N, 105°22'03.6"E, 21 Sep. 2017, *Yahara et. al. V6981* [fr.] (DLU, FU); alt. 1023 m, 21°03'33.7"N, 105°21'39.4"E, 11 Sep. 2016, *Binh et al. V6031* [fr.] (FU).

Distribution. China (Guangdong, Guangxi, Hainan) and Vietnam (Ba Vi National Park, Fig. 3.1).

Ecology in Vietnam. Quercus bella was found on the slopes in evergreen forests in Ba Vi National Park: at alt. 600–1172 m.

Phenology. Flowering from February to April, fruiting from October to December (Huang et al. 1999). Flowering and fruiting specimens were collected from Vietnam in September.

GenBank accession no. Binh et al. V6038: LC331259 (*rbc*L), LC331256 (*mat*K).

Preliminary conservation status. Quercus bella was only previously known as an endemic species to China and distributed in Guangdong, Guangxi and Hainan. The species is not recorded as a threatened species in IUCN (2017). Although only three fruiting individuals of *Q. bella* were

collected in Ba Vi National Park, more individuals are expected to occur there and the habitat in the Ba Vi National Park is currently well-protected from anthropogenic activities under the law. Thus, it is appropriate to place this species under the category Least Concern (LC) following IUCN Red List (IUCN 2012) and Vietnam Red Data book (Ban et al. 2007).

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Legends

Table 3.1. Morphological comparison amongst *Quercus xuanlienensis* Binh, Ngoc & Bon, sp. nov., *Quercus edithiae* Skan and *Quercus fleuryi* Hickel & A.Camus.

Characters	*Q. xuanlienensis*	*Q. edithiae* [1,2,5]	*Q. fleuryi* [3,4,5]
Buds shape	Ellipsoid	Ellipsoid to ovoid	Ovoid
Twigs	Tomentose then glabrous	Densely yellowish brown tomentose when young, later glabrous	Densely orange-brown tomentose when young, later glabrous
Stipules	Linear-lanceolate, 10–14 mm long	Caducous, not seen	Caducous, not seen
Leaf margin	Serrate on upper 1/5–1/7 of lamina	Serrate on the upper 2/3 of lamina	Undulate and serrulate on upper 1/6–1/7 of lamina
Leaf surface	Glabrous on both surfaces	Glabrous on upper surface, reddish brown pubescent on lower surface	Glabrous on both surfaces
Leaf base	Cuneate	Cuneate	Broadly cuneate
Leaf size	(6–)8–11(–15) × 3–4.5(–5) cm	7–15 × 3–5.8 cm	14–22 × 5–9 cm
Length of petioles	1.5–2 cm long	1.7–2.8 cm long	2.5–4 cm long
Number of secondary veins	8–11 pairs	9–10 pairs	10–12 pairs
Infructescence	0.8–1 cm long, each infructescence with (1 or) 2 acorns	0.8–1.5 cm long, each infructescence with (2 or) 3 acorns	0.8–1 cm long, each infructescence with (2 or) 3 acorns
Cupule shape and size	Bowl-shaped, 1.3–1.7 cm high, 1.9–2.1 cm in diam.	Bowl-shaped, 1.5–1.7 cm high, 2.3 cm in diam.	Campanulate to cylindric, 3.6–3.7 cm high, 3.5 cm in diam.
Number of rings on cupule	7–8 rings	6–8 rings	10–13 rings

Margin of rings on cupule	Entire	Almost denticulate except basal 2 or 3 which are entire	Entire
Nut enclosure by cupule	Enclosing 1/5 of the nut	Enclosing 1/4 to 1/3 of the nut	Enclosing 2/3 of the nut
Nut shape and size	Ellipsoid to cylindric-ellipsoid, 5–6 cm high, 2–2.3 cm in diam.	Ellipsoid to cylindrical-ellipsoid, 4–4.5 cm high, 2.1 cm in diam.	Ovoid to cylindrical-ellipsoid, 3–4.5 cm high, 2–3 cm in diam.
Base of the nut	Convex, 9–10 mm in diam.	Slightly convex, ca. 7 mm in diam.	Convex, ca. 12 mm in diam.

[(1)] From the material *Ford 623* (K)

[(2)] From the original description in Hooker's Icon. Pl. 27: t. 2661 1901

[(3)] From the material *Fleury 37831* (P)

[(4)] From the original description in Bull. Mus. Natl. Hist. Nat. 29: 600 1923

[(5)] From the description in flora of China (Huang et al. 1999)

Legends

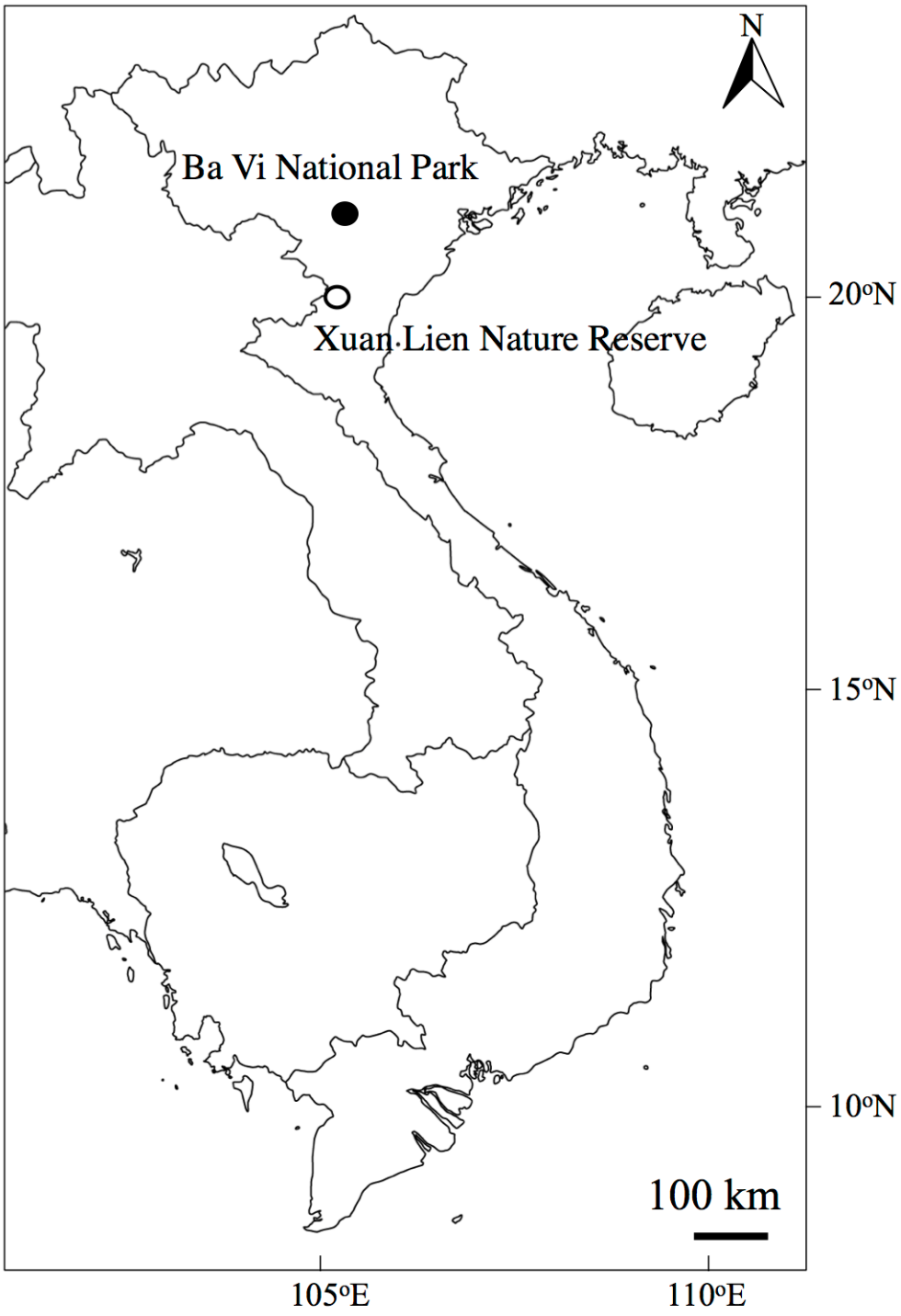

Figure 3.1. Collection sites of *Quercus xuanlienensis* Binh, Ngoc & Bon, *Quercus disciformis* Chun & Tsiang. and *Quercus bella* Chun & Tsiang.

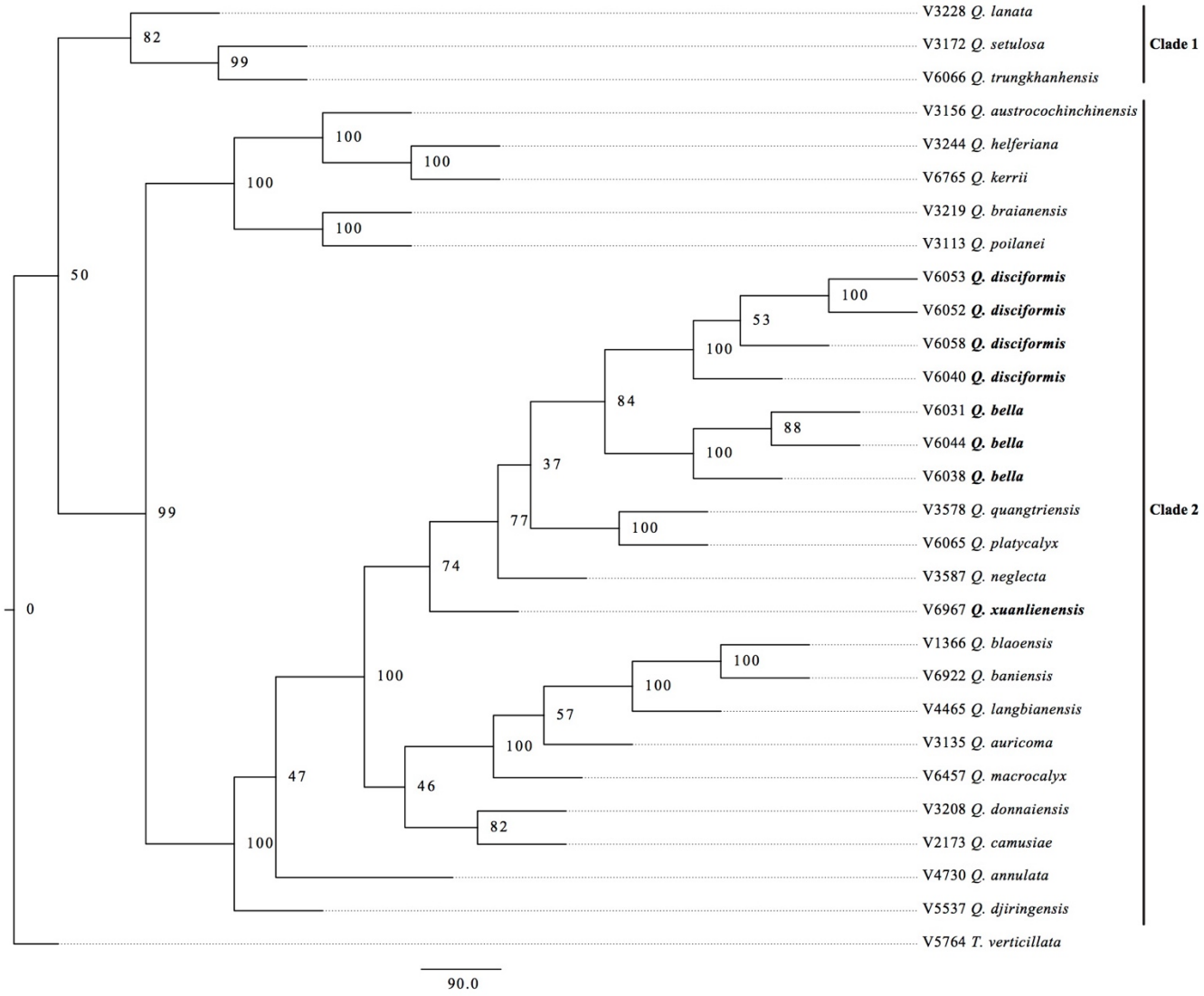

Figure 3.2. NJ tree of 28 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on presence/absence data of 19,916 MIG-seq loci. Branches are labelled with bootstrap support (% of 1000 replicates).

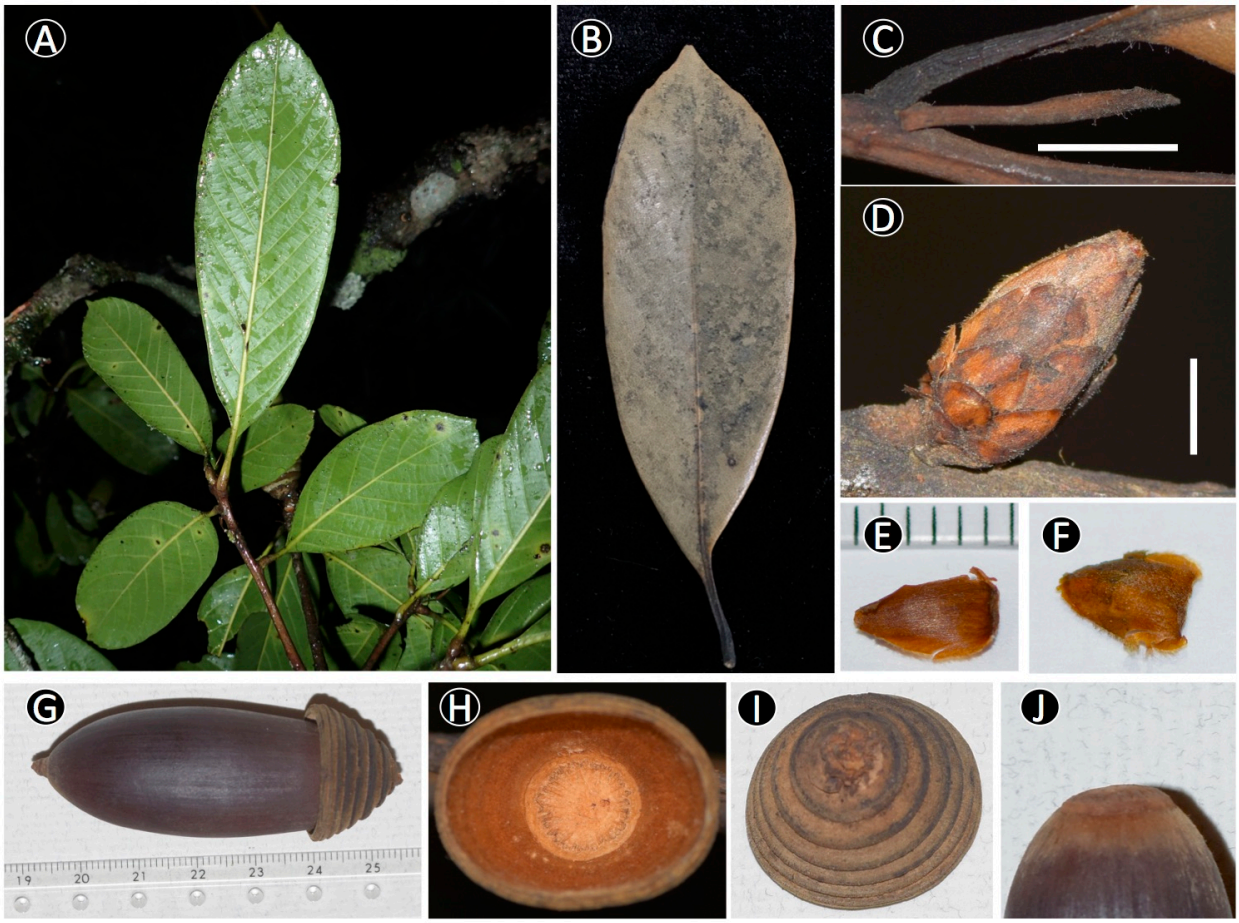

Figure 3.3. *Quercus xuanlienensis* Binh, Ngoc & Bon. **A.** Leafy twig, **B.** Adaxial side of mature leaf, **C.** Stipules, **D.** Bud, **E. & F.** Inside and outside of bud scale, **G.** Mature fruit, **H. & I.** Inside and outside of cupule, **J.** Basal scar of the nut. Scale bars **C**= 5 mm, **D**= 3 mm. Materials from *Binh et al. V6967.*

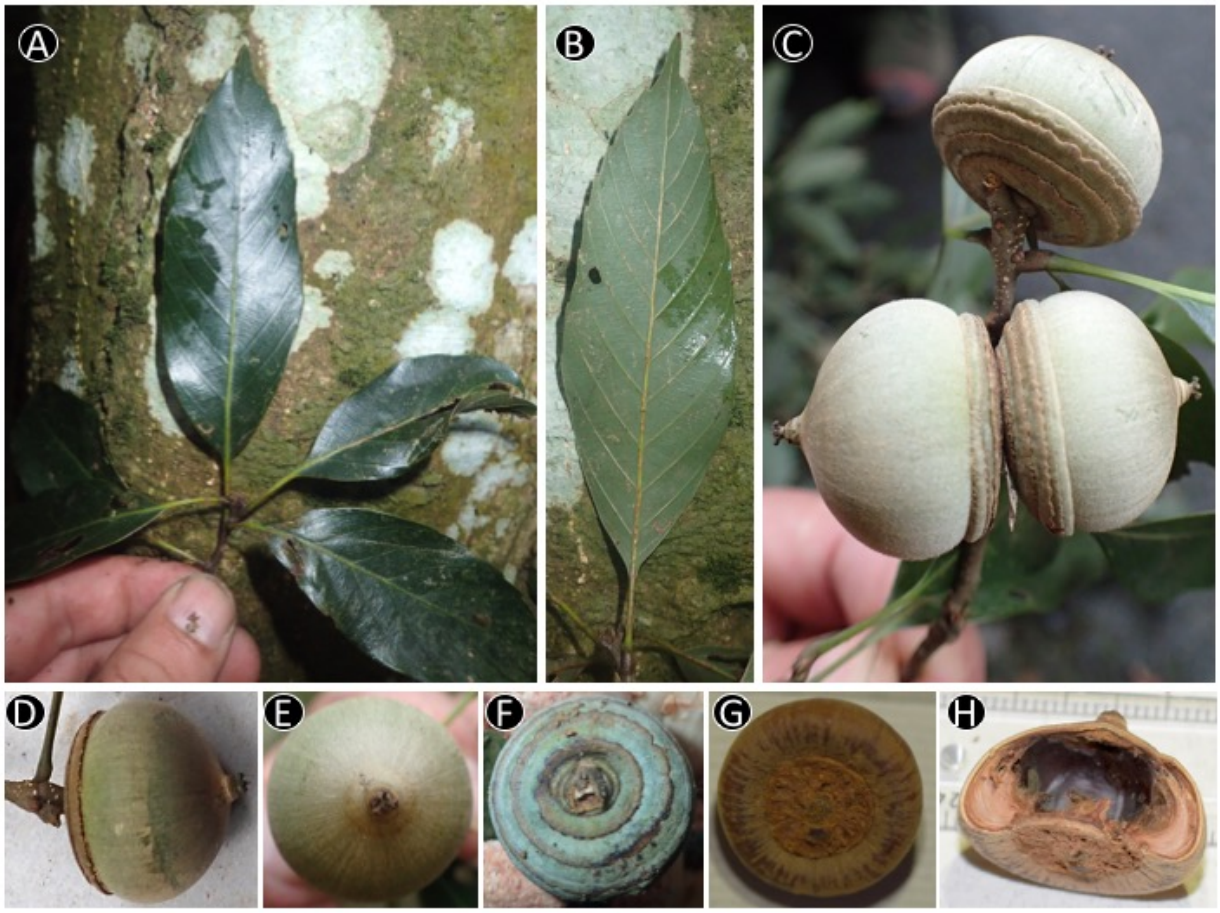

Figure 3.4. Image of *Quercus disciformis* Chun & Tsiang. from *Binh et al. V6058* (FU) **A.** Leafy twig, **B.** Abaxial side of mature leaf, **C-D.** Infructescence and mature fruit, **E.** Nut, **F.** Cupule, **G.** Bottom of nut, **H.** Vertical section of nut.

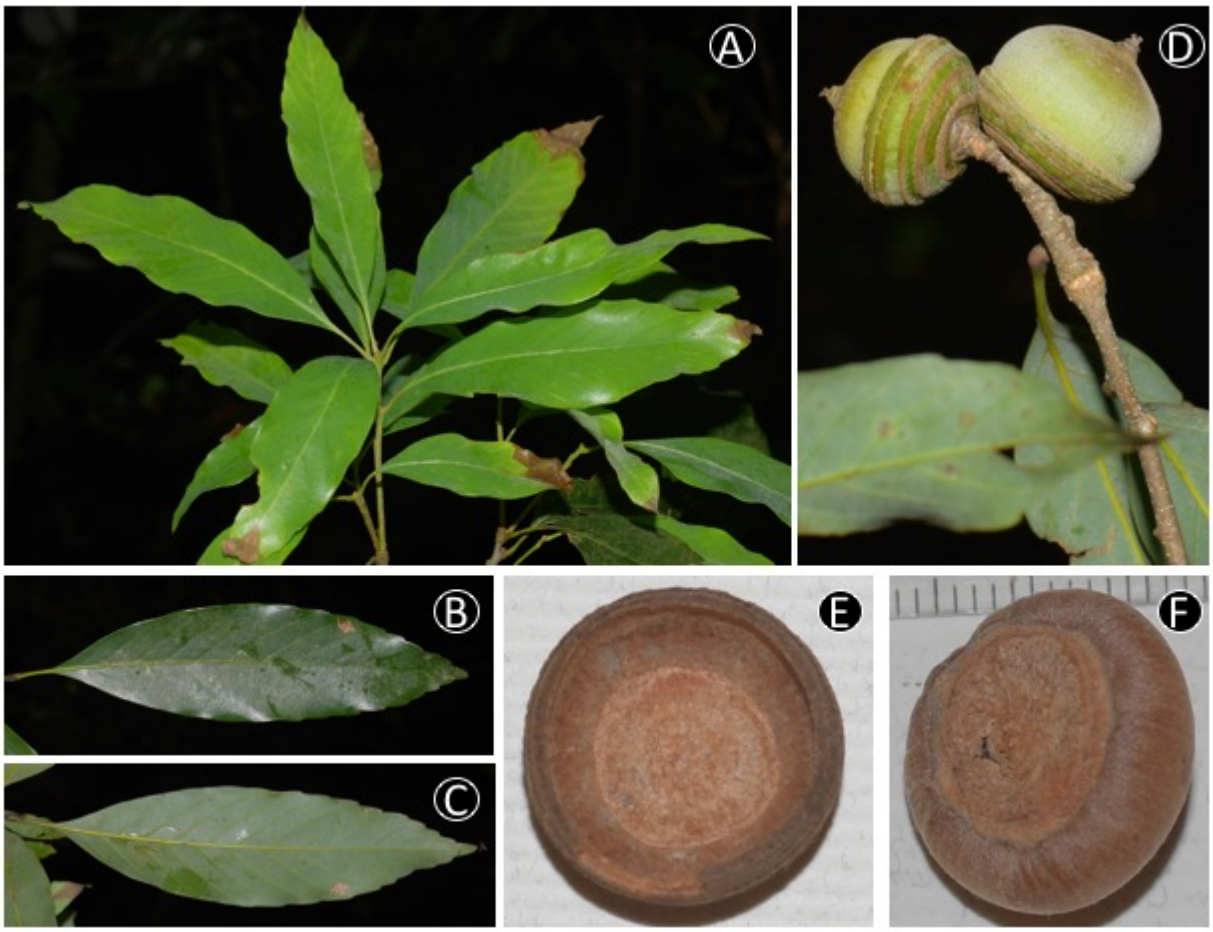

Figure 3.5. Image of *Quercus bella* Chun & Tsiang. **A.** Leafy twig, **B.** Adaxial side of mature leaf, **C.** Abaxial side of mature leaf, **D.** Infructescense and mature fruit (**A-D** from *Yahara et al. V6981* (DLU, FU)), **E.** Inside of cupule, **F.** Bottom of nut (**E-F** from *Binh et al. V6038* (FU)).

Chapter 4. A taxonomic study on 43 species of *Quercus* in Vietnam based on morphology, classic DNA barcodes and genome-wide markers using the next generation sequencing platform.

Abstract

The genus *Quercus* contains about more than 500 species worldwide among which about 125 species were reported from Asia and about 400 species from Americas, Europe, North Africa and Macaronesia. Delimitation of species in the genus *Quercus* has been based on morphological characters mainly by leaves, cupules and acorns. However, those characters often exhibit broad ranges of intraspecific variation most likely due to co-ocurrance of some species in ecologically heterogeneous habitats and their interspecific hybridizations in many species pairs. Some taxonomists successfully used genetic markers to distinguish species in the genus *Quercus*. However, as for genetic markers, widely used DNA barcoding markers such as *rbc*L, *mat*K and ITS do not always provide clear discriminating signals at the species level in *Quercus* because of the existence of paralogous loci. Recently, multiple gene markers and genome-wide markers using the next generation sequencing platform (MIG-seq) have been successfully used in elucidating phylogenetic relationships within the genus *Quercus* and among closely related species. In this study, both three classic barcoding regions and genome-wide markers using the next generation sequencing platform (MIG-seq) were used to clarify the relationships among the closely related species of *Quercus* in Vietnam. Taxonomic revision of the genus *Quercus* in Vietnam is presented based on both molecular and morphological evidence, as well as our field observations. Forty-three *Quercus* species were enumerated in Vietnam, including ten undescribed species tentatively named as *Q. pseudocamusiae*, *Q. fansipanensis*, *Q. haivanensis*, *Q. ngoclinhensis*, *Q. semiundulata*, *Q. sontraensis*, *Q. theifolia*, *Q. tiepii*, *Q. verticillata*, and *Q. vuquangensis*.

Keywords

DNA barcoding, Fagaceae, MIG-seq, *Quercus*, taxonomy, Vietnam.

Introduction

Quercus Linnaeus (1753), with 531 species, is the largest genus of Fagaceae and one of the most widely distributed tree genera (Hubert et al. 2014), In the genus *Querucs*, 250 species are known in the Americas, 125 species in Asia and 156 species in Europe, North Africa and Macaronesia (Govaerts & Flodin 1998, Borazan & Babaç 2003). In the count in the World Checklist and Bibiography of Fagales by Govaerts & Flodin (1998), species richness in Asia is underscored because more recently 104 species (treated 35 species as *Quecus* s. str. and 69 species as *Cyclobalanopsis*) are recorded in the Flora of China (Huang et al. 1999) and 28, 33, 29 and 44 species are recorded in Laos (Newman et al. 2007), Myanmar (Kress et al. 2003), Thailand (Phengklai 2008) and Vietnam (Ho 2003, Ban 2005, Li et al. 2016), respectively. Most of those Asian species of *Quecus* were described until 1930s (Hickel and Camus 1929; Camus 1935, 1935-36, 1936) except in China where more species have been described based on recent collections (Huang et al. 1999). In Vietnam, botanical surveys had a long blank period from 1930s to 2000s when 40 species of *Quercus* were illustrated in An Illustrated Flora of Vietnam by Ho (2003). Since then, *Quercus griffithii* Hook.f. & Thomson ex Miq., *Q. franchetii* Skan, *Q. variabilis* Blume, *Q. nubium* Handel-Mazzetti (Ban 2005) and *Q. lineata* Blume (Li et al. 2016) were newly recorded from Vietnam. More recently, *Q. xuanlienensis* Binh, Ngoc & Bon (Binh et al. 2018a), *Q. trungkhanhensis* Binh & Ngoc (Binh et al. 2018c), *Q. baolamensis* Binh & Ngoc, *Q. bidoupensis* Binh & Ngoc, and *Q. honbaensis* Binh, Tagane & Yahara (Binh et al. 2018b) have been described as new species based on specimens recently collected in Vietnam. Binh et al. (2018a) also newly recorded *Q. bella* Chun & Tsiang and *Q. disciformis* Chun & Tsiang from northern Vietnam and Binh et al. (2018b) showed that *Q. dilacerata* Hickel & A.Camus and *Q. donnaiensis* A.Camus not listed by Ho (2003) and *Q. blaoensis* treated as a synonym of *Q. langbianensis* (Ho 2003) are all distinct species. These findings suggest that there are more new records and undescribed species of *Quercus* in Vietnam. Here, based on morphological and molecular evidence, we show that ten additional species remain to be described.

In *Quercus*, morphological characters of leaves, cupules and acorns have been used to characterize species but those traits often exhibit broad ranges of intraspecific variation (Hubert et al. 2014). This may be because some species occur in ecologically heterogeneous habitats (Dupouey and Badeau 1993) and interspecific hybridizations are often frequent in many species

pairs (Muir et al. 2000, Petit et al. 2004, Hipp et al. 2014). Consequently, the delimitation of *Quercus* species is often very difficult without careful studies using genetic markers (Petit et al. 2004, Binh et al. 2018b; see Chapter 2).

As for genetic markers, commonly used DNA barcoding regions, such as *rbcL*, *matK* and ITS, do not always provide clear discriminating signals at the species level in the genus *Quercus* (Manos et al., 1999, 2001; Piredda et al. 2011). As for ITS, independently generated ITS sequences often gave inconsistent results probably due to the existence of paralogous loci (Mayol & Rosselló 2001; see also Feliner & Rosselló, 2007). By testing sequence orthology, Bellarosa et al. (2004) successfully used ITS sequences for phylogenetic reconstruction of Italian *Quercus* spp. However, more genetic markers are usually required to obtain discriminating signals at the species level in *Quercus* (Binh et al. 2018b; see Chapter 2).

Recently, multiple gene markers (Hubert et al. 2014, Simeone et al. 2016), RAD-seq (Hipp et al. 2014, Cavender-Bares et al. 2015, Fitz-Gibbon et al. 2017, McVay et al. 2017) and MIG-seq (Suyama and Matsuki 2015, Binh et al. 2018b; see Chapter 2) have been successfully used in elucidating phylogenetic relationships within the genus *Quercus* and among closely related species. In this study, we determine sequences of three classic barcoding regions (*rbc*L, *mat*K and ITS) and genome-wide anonymous regions (MIG-seq) to clarify the relationships among the species of *Quercus* in Vietnam. To determine relatively short sequence reads by the next generation sequencer, MIG-seq employs a PCR-based procedure without restriction enzyme digestion steps and is widely applicable than RAD-seq to field samples even with low-quality DNA and/or small quantities of DNA (Suyama and Matsuki 2015).

The purpose of this study is to revise species taxonomy of the genus *Quercus* in Vietnam and examine how may undescribed species are included among the specimens recently collected in a series of our field surveys (Binh et al. 2018b, see Chapter 2) using a standardized belt-transect method that was proposed by Yahara et al. (2012) and has been applied to floristic surveys in Cambodia (Zhang et al. 2016, Tagane et al. 2017) and Vietnam.

Materials and methods

Plant materials and outgroup selection

We studied 91 specimens of the genus *Quercus* collected from 13 conservation areas in Vietnam (including seven National Parks (NP), four Nature Reserves (NR), and two Conservation

areas (CA), Figure 4.1, Table 4.1). In addition, we also compared specimens of *Q. auricoma* sensu Flora of Thailand (Phengklai 2008, *T4287*) and *Q. longistyla* (*T4634*) from Thailand, *Q. cambodiensis* (*C4302*) and *Q. kerrii* (*C7223*) from Cambodia with specimens from Vietnam (indluding *Q. ngoclinhensis* (V6136), *Q. auricoma* (V3135, V3138), *Q. kerrii* (V6677, V6765), *Q. sontraensis* (V6965)). For DNA works, we collected small pieces of fresh leaves from each specimen and dried them with silica gel in Ziplock bags. For some specimens, we collected DNA samples from dried leaves. Voucher specimens were deposited in the Herbarium of Dalat University (DLU), Kyushu University (FU), and partly in the herbarium of Kyoto University Museum (KYO).

According to previous phylogenetic analyses of the family Fagaceae based on internal transcribed spacers (ITS1, ITS2) and nrDNA coding regions for sequences (Manos et al. 2001), the genus *Quercus* is closely related to the genus *Lithocarpus* and *Castanopsis* genus. Therefore, as outgroups for the phylogenetic analysis of MIG-seq, we selected seven species among them collected from Vietnam including *Lithocarpus dahuoaiensis* Ngoc & L.V. Dung, *L. encleisocarpus* (Korth.) A.Camus, *L. rouletii* (Hickel & A.Camus) A.Camus, *L. pachylepis* A.Camus, *L. corneus* (Lour.) Rehder, and *Castanopsis piriformis* Hickel & A.Camus and *C. cerebrina* (Hickel & A.Camus) Barnett. In addition, we used *Trigonobalanus verticillata* Forman as an outgroup following Binh et al. (2018) in both MIG-seq tree and a phylogenetic tree produced by combining the three regions (*rbc*L*, mat*K and ITS; Bayesian tree).

DNA extraction

DNA of each sample was extracted from silica gel dried leaves using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987), with the modifications following Toyama et al. (2015): dried leaf material was milled by QIAGEN TissueLyser to obtain fine powder and washed three times in a 1 ml buffer (including 0.1 M HEPES, pH 8.0; 2% mercaptoethanol; 1% PVP; 0.05 M ascorbic acid). We checked quality and quantity of DNA by NanoDrop (Thermo Scientific).

Amplification and classic DNA sequencing

Two regions of chloroplast genome, maturase K (*mat*K*,* Ford et al. 2009, Cuénoud et al. 2002*)* and ribulose-1,5-biphosphat carboxylase oxygenase (*rbc*L*,* Levin et al. 2003, Fay et al.

1997) and one nuclear ribosomal DNA, internal transcribed spacer (ITS, Rohwer et al. 2009), were used in this study (Table 4.2). Polymerase chain reaction (PCR) for all regions were conducted in a volume of 10 µl solution, consisting of 1 µl template DNA, 3.25 µl of Milli-Q water (Millipore), 0.5 µl 2xGflex PCR Buffer (Mg2+, DNTP plus), 0.3 µl of each forward and reverse primer (10 pM) and 0.15 µl Tks Gflex DNA polymerase (1.25 unit/µl). The PCR reactions were performed using the following cycling conditions: for *rbc*L and ITS, template denaturation at 95°C for 4 min, followed by 29 (for *rbc*L) and 35 (for ITS) cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min, and extension at 72°C for 1 min and a final extension of 10 min at 72°C; for *mat*K, template denaturation at 94°C for 5 min, followed by 38 cycles of denaturation at 94°C for 40 sec, annealing at 48°C for 40 sec, and extension at 72°C for 40 sec and a final extension of 7 min at 72°C. Each 5 µl of PCR products was purified with 0.5 µl ExoSap-IT enzyme and 1.6 µl of Milli-Q water (Millipore), and incubated at 37°C for 30 min and subsequently at 80°C for 15 min for deactivation of the enzyme. DNA sequences were determined using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Amplification and next generation DNA sequencing – MIG-seq

Primers designed for "multiplexed ISSR genotyping by sequencing" (MIG-seq, Suyama and Matsuki 2015) were used to amplify short sequences (loci) from each genome of the 94 DNA samples of the genus *Quercus* and an outgroup *Trigonobalanus*. We performed sequencing following the protocol of Suyama and Matsuki (2015) with minor modifications described in Binh et al. (2018b). In the 1st PCR, ISSR regions from genomic DNA were amplified with MIG-seq primer set-1 (Appendix). For amplification, 7 µl master mix contained 1 µl of DNA template, 3.5 µl of 2x Multiplex PCR Buffer, 0.14µl of 1st PCR primers (10 µM), 0.035 µl of Multiplex PCR Enzyme mix and 0.225 µl of deionized water. Reactions were performed using the following cycling conditions: template denaturation at 94°C for 1 min, followed by 25 cycles of denaturation at 94°C for 30 sec., annealing at 38°C for 1 min, and extension at 72 °C for 1min; with an additional final extension at 74 °C for 10 min. Quality of 1st PCR amplified products of each sample was checked by a Microchip Electrophoresis System (MultiNA, Shimadzu) with the DNA-2500 Reagent Kit (Shimadzu). Then, each 1st PCR product was diluted 50 times with deionized water. 2nd PCR was performed using indexed primers of each sample. For amplification, 17 µl PCR

Reaction Mix included 2.5 µl of diluted 1st PCR product, 2.4 µl of 5x PrimeSTAR GXL Buffer, 0.96 µl of dNTP mixture, 1.2µl of 2nd PCR forward primer (2 µM), 1.2µl of 2nd PCR reverse primer (2 µM), 0.24 µl of PrimerSTAR GXL polymerase, and 3.5 µl of deionized water. Reactions were followed by 20 cycles of denaturation at 98°C for 10 sec., annealing at 54°C for 15 sec., and extension at 68°C for 1 min. Then, 3 µl of each 2nd PCR product was pooled as a single mixture library. The mixture was purified, and fragments in the size range 350–800 bp were selected by a Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA). Finally, the concentration of size-selected library was measured by using a SYBR green quantitative PCR assay (Library Quantification Kit; Clontech Laboratories, Mountain View, CA, USA) with approximately 10 pM of libraries that were used for sequencing on an Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 cycle, Illumina).

Phylogenetic analyses

A phylogenetic tree combining the three regions (*rbc*L*, mat*K and ITS) was constructed by the Bayesian method using 52 samples from Vietnam and another sample from Cambodia (*Q. cambodiensis*, C4302). In this study, we newly determined DNA sequences of 26 samples (including 74 sequences), and the rest of 106 sequences were obtained from GenBank (comprising sequences of outgroup, Table 4.1). The sequences were aligned by MEGA v7.0 (Kumar et al. 2016). A Bayesian method implemented in the program BEAST v1.8.4 (Drummond et al. 2012) was used to construct phylogeny as Binh et al. (2018, see chapter 3).

For constructing a MIG-seq tree, a raw MIG-seq data set of 105 samples was pretreated to control the data quality following the procedure of Suyama and Matsuki (2015) as described in Binh et al. (2018b) (Chapter2, Chapter 3). After, we constructed MIG-seq by neighbor-joining method as described in Binh et al. (2018b, see Chapter 2, Chapter 3). A total of 35,259 loci of 95 *Quercus* samples were used for the final phylogenetic tree.

Morphological observations

After distinguishing species based on the molecular evidence (MIG-seq and Bayesian trees), we carefully compared their morphological traits with available type specimens or authentic

specimens of all the *Quercus* species described from Vietnam, China, Cambodia, Laos and Thailand by visiting the herbaria HN, P, VNM, FU, DLU and using digital images of specimens on JSTOR Global Plants (https://plants.jstor.org/) and the websites of various herbaria. Moreover, we also reviewed original description of each species.

Results

PCR amplification and sequencing

Anonymous sequences of all 95 samples were successfully amplified with primers designed for MIG-seq (Suyama and Matsuki 2015). However, ITS sequences of some samples were not successfully amplified, most likely due to the low DNA quality (Table 4.1). Therefore, the phylogenetic tree combining the three regions (*rbc*L, *mat*K and ITS; Bayesian tree) included only 53 samples of *Quercus*, while we used all 95 samples to construct a MIG-seq tree.

Phylogenetic analysis of combined data

Sequence characteristics

Among the species of *Quercus* compared, the DNA sequences of maturase K (*mat*K), ribulose-1,5-biphosphat carboxylase oxygenase (*rbc*L), and internal transcribed spacer (ITS) varied in length from 762 bp (*Q. quangtriensis*) to 921 bp (*Q. camusiae* and *Q. honbaensis*), 569 bp (*Q. camusiae*) to 727 bp (*Q. honbaensis* and *Q. setulosa*), and 413 bp (*Q. pseudocamusiae*) to 678 bp (*Q. annulata* and *Q. songtavanensis*), respectively. The data matrix of the two cpDNA regions included 834 characters; 47 sites of *mat*K and 12 sites of *rbc*L were variable among 54 samples (see Table 4.3 for details of the statistics). The aligned data matrix of nuclear ITS sequences comprised 543 characters; 142 sites were variable among 54 samples (Table 4.3). A total aligned length combining *rbc*L, *mat*K and ITS were 2,034 bp. Total parsimony-informative and variable characters were 78 and 204, respectively (Table 4.3).

Phylogenetic analyses

In the Bayesian tree based on the sequences of three regions (Fig. 4.2A, 4.2B), *Quercus* species were clustered to two clades (clade 1 and clade 2), each supported by Bayesian posterior probability 1.00. Clade 1 included two species of the subgenus *Quercus, Q. setulosa* and *Q. trungkhanhensis,* and clade 2 included 30 species of the subgenus *Cyclobalanopsis.* Among the

latters, 24 species are previously described species whereas six are undescribed species. In Clade 2, *Q. verticillata* was sister to all the other species that were clustered to Clade 2A1 (PP=1.00) comprising *Q. austrocochinchinensis, Q. helferiana,* and *Q. kerrii* and Clade 2A2 (PP=0.79) comprising the other 25 species. In Clade 2B2, *Q. tiepii* was sister to the other 24 species in which a clade including *Q. braianensis* and *Q. poilanei* is sister to a clade comprising the other 22 species. In species in which two or more samples were sequenced, monophyly of a species was supported by PP = 1.00 for *Q. annulata*, *Q. austrocochinchinensis*, *Q. baniensis*, *Q. braianensis* and *Q. langbianensis*, 0.99 for *Q. helferiana* and *Q. sessilifolia*, 0.88 for *Q. kerrii*, 0.87 for *Q. neglecta*, and 0.37 for *Q. honbaensis*. *Quercus poilanei* was not monophyletic if *Q. braianensis* was distinguished based on morphology.

A phylogenetic tree using MIG-seq

The MIG-seq tree (Figure 4.3A-B) indicates that 45 species of *Quercus* are clustered to two clades with 97% and 93 % bootstrap value, respectively. Clade M1 (Bootstrap value, 87%) included three species of the subgenus *Quercus*: *Q. lanata, Q. setulosa* and *Q. trungkhanhensis* (Fig. 4.3A), and clade M2 (Fig. 4.3A, 4.3B) included 42 species of the subgenus *Cyclobalanopsis* including 32 described and 10 undescribed species. In clade M2, *Q. tiepii* (*V4285*) is sister to all the other species that are clustered to two clades; Clade A and Clade B supported by 100 % and 91 % bootstrap values, respectively. Clade A was clustered to two clades, Clade A1 and Clade A2, both supported by 100 % bootstrap value. Clade A1 included *Q. helferiana, Q. kerrii and Q. austrocochinchinensis* while Clade A2 included *Q. poilanei and Q. braianensis*. Clade B was clustered to two clades, Clades B1 and B2, both supported by 100 % bootstrap value. Clades B1 included *Q. sessilifolia, Q. fansipanensis, Q. semiundulata, Q. theifolia and Q. bidoupensis.* Clade B2 included the other 32 species. Among the 32 species, there were four clades supported by bootstrap values largher than 80%: (1) a clade including *Q. disciformis, Q. bella, Q. quangtriensis, Q. platycalyx* and *Q. chrysocalyx* supported by 95% bootstrap value, (2) a clade including *Q. camusiae, Q. donnaiensis, Q. sontraensis, Q. cambodiensis and Q. haivanensis* supported by 82% bootstrap value, (3) a clade including *Q. honbaensis, Q. baolaensis,* and *Q. longistyla* supported by 87% BP, and (4) a clade including *Q. vuquanensis and Q. blakei* supported by 100 % bootstrap value. The MIG-seq tree included 22 species in which two or more samples were examined. Among them, monophylies of 17 species were supported by a bootstrap

value larger than 90%: 100% in *Q. annulata*, *Q. auricoma*, *Q. austrocochinchinensis, Q. bella*, *Q. braianensis, Q. poilanei, Q. cambodiensis*, *Q. camusiae*, *Q. djringensis*, *Q. disciformis*, *Q. honbaensis*, *Q. neglecta* and *Q. xanthoclada*, 99% in *Q. donnaiensis* and *Q. helferiana*, 96% in *Q. langbianensis* and *Q. sessilifolia* and 92% in *Q. vuquangensis*. On the other hand, monophylies of *Q. kerrii* and *Q. macrocalyx* were supported only by 54% and 64%, respectively. The monophyly of *Q. bidoupensis* was not supported because a clade including *Q. semiundulata*, *Q. theifolia* and V4328 identified as *Q. bidoupensis* was supported by 71% bootstrap value and it was sister to V3202, another sample identified as *Q. bidoupensis*. Besides, the monophyly of *Q. baniensis* was also not supported because a clade comprising *Q. auricoma, Q. blaoensis, Q. chevalieri, Q. langbianensis, Q. verticillata*, V6922 identified as *Q. baniensis* was supported by 35% bootstrap value. Another samples (V3089) is weakly related to these six species (22% bootstrap value) that is also identified as *Q. baniensis.*

Morphological observations

Based on morphological observations on 91 specimens of *Quercus* collected from Vietnam, we identified 43 species among which the following ten species are considered to be undescribed; *Q. semiundulata* (*V6597*), *Q. theifolia* (*V6618*), *Q. haivanensis* (*V3042*), *Q. ngoclinhensis* (*V6136*), *Q. pseudocamusiae* (*V3572*), *Q. tiepii* (*V4285*), *Q. verticillata* (*V4365*), *Q. sontraensis* (*V6965*), *Q. vuquangensis* (*V5724, V5927*), *Q. fansipanensis* (*V5101*). Diagnostic characters of those undescribed species are described below by comparing those species with other species having similar morphological traits and/or close phylogenetic positions on the MIG-seq tree. Evidence of sympatric occurrence with related species is also provided if it is the case.

Quercus semiundulata, Q. theifolia and *Q. ngoclinhensis* were collected at alt. 1,354 m, 1,070 m and 1,168 m of Ngoc Linh Nature Reserve. The MIG-seq tree showed that *Q. semiundulata* and *Q. theifolia* were sister to each other, but *Q. semiundulata* is distinct form *Q. theifolia* in having leaves with undulate and entire margin (vs. distinctly serrate along the upper half of leaf margin in *Q. theifolia*). *Quercus semiundulata* is similar to *Q. bidoupensis* in lanceolate leaves undulate along margin, but leaves are almost entire (vs. distinctly serrate along the upper half of leaf margin in *Q. bidoupensis*). *Quercus theifolia* is also similar to *Q. bidoupensis* in lanceolate leaves distinctly serrate along upper half of margin, but leaves are smaller (5–9 x 2–3 cm vs. 10–13 x 2.5–4 cm), thicker and not undulate. *Quercus ngoclinhensis* is distinct from *Q.*

semiundulata and *Q. theifolia* in leaf margin distinctly serrate in upper 1/2–1/3, young leaves dark red and densely white hairy (vs. margin undulate and almost entire in *Q. semiundulata;* young leaves glabrous in both *Q. semiundulata* and *Q. theifolia*). *Quercus ngoclinhensis* is more similar to *Q. longistyla* (type specimen from Thailand, Phengklai 2008) in having leaves lanceolate or elliptic-lanceolate, margin serrate along upper 1/3, young shoot and young leaves white densely hairy, but is distinguished in leaf base broadly acute and young leaves dark red (vs. base acute, young leaves green). Among Vietnamese species, it is most similar to *Q. blaoensis* in having lanceolate or elliptic-lanceolate, margin serrate, young shoot densely with hairy, but is distinguished in leaf margin distinctly serrate in upper 1/2–1/3, base broadly acute, apex acuminate or caudate, young leaves dark red (vs. leaves margin distinctly serrate in upper 1/3, base cuneate or oblique, apex acute, young leaves green), and petioles shorter (0.5–1 cm vs. 0.9–1.8 cm in *Q. blaoensis*), but *Q. blaoensis* is not sister to *Q. ngoclinhensis* on the MIG-seq tree.

Quercus haivanensis was collected at alt. 663 m of Hai Van Pass, Dang Nang Province. This species is similar to *Q. arbutifolia* (described from Nha Trang, Khanh Hoa Province at alt. 1,700 m, not collected in this study) in having entire leaves rounded at the tip, but different in elliptic leaf blades (vs. obovate to ovate in *Q. arbutifolia*), chartaceous leaf texture (vs. coriaceous), flat leaf margin (vs. recurved leaf margin), and longer petioles (1.5 – 1.8 cm vs. 0.2 – 0.5 cm in *Q. arbutifolia*).

Quercus pseudocamusiae and *Q. vuquangensis* were collected from Vu Quang National Park at alt. 407 m and 1,335 m, respectively. MIG-seq tree showed that two species are not close to each other; *Q. pseudocamusiae* is weakly related to *Q. camusiae*, whereas *Q. vuquangensis* is sister to *Q. blakei*. In leaf morphology, *Q. pseudocamusiae* is more similar to *Q. camusiae* in lanceolate to elliptic leaf blades and almost entire leaf margin, but distinct from *Q. camusiae* in leaves cuspidate at apex (vs. acute in *Q. camusiae*), young shoot and petioles white hairy (vs. golden hairy in *Q. camusiae*). Both *Q. vuquangensis* and *Q. blakei* are collected from Vu Quang National Park, but the former was collected at 1,335 m while the latter was collected at 650 m alt. *Quercus vuquangensis* and *Q. blakei* are easily distinguished in leaves (decurrent at base vs. cuneate at base; leaf blades oblong-elliptic to lanceolate, 11–19 x 2–4 cm vs leaf blade ovate-elliptic to lanceolate 7–11 x 1.5–2.8 cm, leave margin serrate along upper 1/3 vs. serrate along upper 2/3). *Quercus vuquangensis* is more similar to *Q. chapensis* (described from Chapa, Lao Kai Province, not collected in this study) in leaf blades oblong-elliptic to lanceolate with sharp

serrations, apex acuminate, but different in thinner leaves, base decurrent, leaf margin serrate along upper 1/3, and petioles 0.5 – 1 cm (vs. thicker leaves, base broadly cuneate, leaf margin serrate along upper 2/3, and petioles 1 – 2.5 cm).

Quercus tiepii and *Q. verticillata* were collected from Bidoup - Nui Ba National Park, Lam Dong Province, at alt. 1,800 m and 1,698 m, respectively. MIG-seq tree showed that both species have any close relative. In leaf morphology, *Q. tiepii* is similar to *Q. ramsbottomii* distributed in Myanmar (described fromTennasserim at alt. 1,800–1,950 m, not collected in this study) and western Thailand in ovate to ovate-lanceolate leaves with apex acute or obtuse, but different in leaf margin entire (vs. serrate in the upper half or rarely entire in *Q. ramsbottomii*), and petioles glabrous, 0.5 – 1.3 cm (vs. petioles tomentose then glabrescent, 1.5 – 2.5 cm in *Q. ramsbottomii*). *Quercus tiepii* is also similar to *Q. camusiae* in having entire leaf margin, but distinguished in leaf shape ovate to ovate-lanceolate (vs. lanceolate to elliptic in *Q. camusiae*) and young shoot and petioles glabrous (vs. young shoot and petioles golden hairy). *Quercus verticillata* is similar to *Q. camusiae* and *Q. pseudocamusiae* in lanceolate to elliptic leaves almost entire along margin, having secondary vein 10 – 14 pairs, but young shoot and petioles are glabrous (vs. young shoot and petioles hairy in *Q. camusiae* and *Q. pseudocamusiae*).

Quercus sontraensis (*V6965*) was collected at alt. 200 – 300 m from Son Tra Conservation areas. MIG-seq tree showed that this species is weakly related to *Q. cambodiensis*, *Q. camusiae*, *Q. donnaiensis* and *Q. haivanensis*. Among those species, *Q. sontraensis* is most similar to *Q. cambodiensis* in lanceolate to elliptic leaves, cupules cup-shaped with 7 – 8 rings and densely golden hairy, nut subglobose and densely golden hairy, but different from the latter in having leaf margin distinctly serrate in upper 1/3 (vs. leaf margin almost entire or with a few low teeth in upper 1/4), and cupule bract margin distinctly toothed in all rings (vs. distinctly toothed in two lower rings in *Q. cambodiensis*). In the MIG-seq tree, this species is sister to *Q. donnaiensis* with 66% bootstrap value. However, *Q. sontraensis* is distinct from *Q. donnaiensis* in the cupule with 7 –8 rings, enclosing <1/2 of the nut, bract margin distinctly toothed in all rings, nut densely hairy, and nut scar flat (vs. 5 – 6 rings, enclosing 1/2 of the nut, bract margin entire, nut sparsely hairy, nut scar convex in *Q. donnaiensis*).

We collected the following three *Quercus* species from Fansipan mountain, Hoang Lien National Park, Lao Cai Province: *Q. annulata* (*V4730, V4801), Q. sessilifolia* (*V5047, V5112*) and *Q. fansipanensis* (*V5101)*. MIG-seq tree showed that *Q. fansipanensis* and *Q. sessilifolia* are sister

to each other, but *Q. annulata* is not close to them. Among them, *Q. fansipanensis* and *Q. sessilifolia* were collected in the same stand at alt. 2,225 m where two species were easily distinguished in leaf shape (ovate-lanceolate or oblong-elliptic, 6–10 cm x 2.5–3.5 cm, margin undulate in upper 1/3 margin, base broadly cuneate to subrounded in *Q. fasipanensis* vs. linear lanceolate, 6–12 cm x 1.5–2.3 cm, margin entire, base cuneate in *Q. sessilifolia*). *Quercus fansipanensis* is more similar to *Q. annulata* in having leaves ovate-lanceolate or oblong-elliptic, apex acuminate to caudate, base broadly cuneate to subrounded, but different in having leaf blades glabrous on both surfaces and undulate in upper 1/3 margin (vs. leaf blade covered with prostrate simple hairs on the lower surface, glabrous on the upper surface, leaf margin sharply serrate in upper 2/3, tooth often awn-like in *Q. annulata*).

Discussion

Evidence from the molecular phylogenies and morphological observations supported that our collections include 10 undescribed species. Among them, *Q. tiepii* is not closely related to any other Vietnamese species both in the three genes tree and in the MIG-seq tree, and morphologically distinct. For *Q. fansipanensis, Q. haivanensis, Q. semiundulata, Q. theifolia* and Q. *vuquangensis*, the MIG-seq tree strongly supports the sister relationships between the following four pairs: (1) *Q. fansipanensis* and *Q. sessilifolia* (supported by 100% BS), (2) *Q. haivanensis* and *Q. cambodiensis* (41%), (3) *Q. semiundulata*+*Q. theifolia* and *Q. bidoupensis* (71%) and (4) *Q. vuquangensis and Q. blakei* (100%). In the three genes tree, (1) *Q. fansipanensis* is not included, (2) the monophyly of a clade including *Q. haivanensis* and *Q. cambodiensis* is supported only by 0.22 posterior probability, (3) *Q. semiundulata* is sister to *Q. bidoupensis* while *Q. theifolia* is not included, and (4) *Q. vuquangensis* and *Q. blakei* belong to the same clade supported by 1.00 PP but not sister to each other. Thus, the relationships supported by the MIG-seq tree and the three genes tree are not always consistent. However, morphological obesrvations supported that the five species are distinguished by some diagnostic traits from any of their candidate sister species suggested from the MIG-seq tree or the three genes tree.

Among the other three species, *Q. pseudocamusiae* belongs to a clade of the MIG-seq tree supported by 28 % bootstrap value including *Q. cambodiensis, Q. camusiae, Q. donnaiensis, Q. haivanensis* and *Q. sontraensis*. In the three genes tree, *Q. pseudocamusiae* belongs to a well-supported clade (1.00 PP) including *Q. blakei, Q. chrysocalyx, Q. neglecta, Q. vuquangensis*, and

Q. xanthoclada. Thus, the positions of *Q. pseudocamusiae* on two phylogenetic trees are not consistent again but both suggest that *Q. pseudocamusiae* is not close to any particular species. Also, the MIG-seq tree showed that the other two species are not closely related to any other species; *Q. sontraensis* is only weakly related to *Q. donnaiensis* and *Q. ngoclinhensis* is only weakly related to a clade including *Q. auricoma, Q. baniensis, Q. blaoensis, Q. chevalieri, Q. langbianensis,* and *Q. verticillata*.

The inconsistencies between the two phylogenetic trees could be resulted from the following factors. First, it has been known that *rbc*L, *mat*K and ITS are not always reliable markers for reconstructing the phylogeny of *Quercus* due to lower divergence rates of *rbc*L and *mat*K and the existence of paralogous genes in ITS (Manos et al., 1999, 2001; Mayol & Rosselló 2001; Feliner & Rosselló, 2007; Piredda et al. 2011). Those limitations of *rbc*L, *mat*K and ITS sequences may be a factor contributing the inconsistencies. While the sequences of *rbc*L, *mat*K and ITS provided 2,034 bp aligned sequence length, 78 parsimony-informative sites and 204 bp mutations, MIG-seq provided the presence/absence data of 35,259 loci. Because the larger dataset was used for the phylogenetic reconstruction, the MIG-seq tree is considered to be more reliable than the three genes tree. Second, it is well known that hybridizations often result in inconsistencies between trees reconstructed by plastid and nuclear DNA sequences (Soltis and Kuzoff, 1995; Kellogg et al., 1996; Sang & Zhong 2000; Acosta & Premoli 2010) and hybridizations is common in *Quercus* at least in Europe (Muir & Schloetterer 2005, Viscosi et al. 2009) and North America (Veblen et al. 1996, Tovar-Sánchez & Oyama 2004, González-Rodríguez et al. 2004). Also in Vietnam, intrespecific hybridizations may have contributed to the evolution of Quercus and resulted in inconsistencies between cpDNA and nuclear DNA phylogenies. In the case when hybridization occurred and chloroplast genomes were transferred between species, nuclear genome markers provide more reliable evidence for the phylogenetic relationship (check Sang & Zhong 2000; Acosta & Premoli 2010). In this point, the MIG-seq tree is considered to be more reliable. Third, we could not determine seqences of *rbc*L, *mat*K and ITS for 19 samples of 13 species that are included in the MIG-seq tree, probably due to low quality of DNA samples for those species. This smaller sample size may lower the resolution of the three genes tree compared with the MIG-seq tree. In conclusion, the MIG-seq tree may be more reliable than the three genes tree, although we need further studies using other genetic markers to obtain a more reliable phylogenetic tree of *Quercus* in Vietnam.

In addition, among 22 species in which two or more samples were included in the MIG-seq tree, 19 species (*Q. vuqunagensis* and 18 described species) were monophyletic, demonstrating that MIG-seq provides reliable genetic markers to circumscribe a species. In two other species, *Q. bidoupensis* and *Q. baniensis*, monophyly was not supported. First, two samples of *Q. bidoupensis* are paraphyletic because we treated *Q. semiundulata* and *Q. theifolia* as different species. The latter two species could have been dereived from a lineage of *Q. bidoupensis* migrated to Ngoc Linh where *Q. semiundulata* and *Q. theifolia* were diverged. If this is the case, "*Q. bidoupensis*" includes a pair of ancestral and derived lineages that could be treated as different species by further careful studies. Second, two samples of *Q. baniensis*, V3089 and V6922, are polyphyletic in the MIG-seq tree in spite that the monophyly of V3089 and V6922 was strongly supported (PP = 1) in the three genes tree. It is notable that PP values supporting internodes separating V3089 and V6922 are very low, varying from 22 to as high as 41. This low resolution could be due to interspecific hybridization e.g. between *Q. baniensis* and *Q. auricoma* which we collected in Ba Na National Park and its vicinity, Son Tra. To test this possibility, we need further studies using more samples to conclude the relationship between V3089 and V6922.

We identified the other 15 species for which mophophilies were supported by the MIG-seq tree as *Q. annulata, Q. auricoma, Q. austrocochinchinensis, Q. braianensis, Q. camusiae, Q. djiringensis, Q. helferiana, Q. kerrii, Q. lanata, Q. langbianensis, Q. macrocalyx, Q. neglecta, Q. poilanei, Q. sessilifolia,* and *Q. xanthoclada,* based on comparisons of our collections with the type specimens. Among them, 13 species except *Q. sessilifolia* and *Q. xanthoclada* were illustrated by Ho (2003) in which *Q. neglecta* was listed as *Q. bambusaefolia* Hance. We also identified the other eight species for which only single each specimen was included in the MIG-seq tree as *Q. augustinii, Q. blakei, Q. chevalieri, Q. chrysocalyx, Q. platycalyx, Q. quangtriensis, Q. setulosa,* and *Q. songtavanensis.* Among them, *Q. platycalyx* treated as a synonym of *Q. quangtriensis* by Ho (2003) was confirmed to be distinct from the latter. We collected both species in Vu Quang National Park where two species were clearly differentiated in cupule and leaf morphologies. The remaining seven species were illustrated by Ho (2003). In conclusion, we confirmed the distinctiveness of 43 species including 10 undescribed and 33 described species. Taxonomic notes on each species are given in the taxonomic treatments in case those are necessary.

Conclusions

In this study, the taxonomy of *Quercus* in Vietnam is revised based on molecular analyses and morphological observations of 91 specimens we recently collected in the field. As a result, we identified 43 *Quercus* species including ten undescribed species, five recently described species (Binh et al. 2018a, b, c), six species not listed by Ho (2003) but confirmed as distinct by Binh et al. (2018b; *Q. blaoensis*, *Q. dilacerata* and *Q. donnaiensis*) or in this study (*Q. baniensis, Q. platycalyx, Q. sessilifolia* and *Q. xanthoclada*), two species recently reported from Vietnam (Binh et al. 2018a; *Q. bella* and *Q. disciformis*), and the remaining 20 species illustrated by Ho (2003). Consequently, the species of *Quercus* in Vietnam rose from 40 (Ho 2003) to 59 species. This figure shows that Vietnam is the center of species diversity of *Quercus* in Asia next to China where 104 species are recorded in the Flora of China (Huang et al. 1999) and an additional species was reported more recently (Li et al. 2016). Considering the significant increase of the number of the species by our recent studies suggests that even more species of *Quercus* could be discovered from Vietnam by further field surveys. Also, we need to collect 16 species among the 59 total in order to confirm their identities by the MIG-seq analysis. Below, we list the 59 species of *Quercus* including three species for which we have never examined specimens and their occurrences in Vietnam needs further carful examinations.

Taxonomic treatments for previously described species in Vietnam

***Quercus annulata* Sm.**, Cycl. 29: 22 (1819); Ho, Ill. Fl. Vietnam 2: 656, (2003), as *"Quercus annulata* Wall." — *Quercus glauca* subsp. *annulata* (Sm.) A.Camus, Chênes Texte 1: 287 (1938); Ban, List Pl. Vietnam 2: 265 (2005). — *Cyclobalanopsis annulata* (Sm.) Oerst, Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn 18: 78 (1866); Huang et al., Fl. China 4: 396 (1999).

Type: NEPAL. *Wallich 2767* (E [E00301167, image!], K [K001117053, image!]).

Specimens examined: VIETNAM. Lao Cai Province, Hoang Lien National Park, Mt. Fansipan, 22°20'57.1"N, 103°46'15.4"E, alt. 1900 m, 30 April 2016, *Binh et al. V4730* [ster.] (DLU, FU!), *Ngoc et al. V4801*[ster.] (DLU, FU!).

Distribution: Nepal, India, China (W Sichuan, E Xizang, W to SW Yunnan), Vietnam (Lao Cai).

Phenology: Flowering from March to April, fruiting from October to November.

Ecology: Broad-leaved evergreen forests, lower montane evergreen forest, along nature trail.

Vernacular name: Sồi vòng.

***Quercus augustinii* Skan**, J. Linn. Soc., Bot. 25: 507 (1899); Phangklai, Fl. Thailand 9(3): 361 (2008). —*Quercus augustinii* Skan, Ho, Ill. Fl. Vietnam 2: 656 (2003); Ban, List Pl. Vietnam 2: 262 (2005). —*Cyclobalanopsis augustinii* (Skan) Schottky, Bot. Jahrb. Syst. 47: 656 (1912); Huang et al., Fl. China 4: 389 (1999); Phengklai, Fl. Thailand 9 (3): 361 (2008).

Type: CHINA. Yunnan, *Henry 11430* (K [K000832095, image!]).

Specimens examined: VIETNAM. Lao Cai Province, Mt. Fansipan, alt. 3000 m, *Poilane 17128* (P [P06852103, image!]); Dak Lak Province, Mt. Chu Yang Sin, alt. 2000 m, 24 Apr. 1941, *Poilane 32565* [young fr.] (P [P06852106, image!], VNM [VNM00022825!]); Da Nang Province, Ba Na Nature Reserve, 15°59'46.49"N, 108°00'02.88"E, alt. 1233 m, 29 May 2015, *Tagane et al. V3106* [ster.] (FU!, DLU).

Distribution: This species occurs in China (Guangxi, Guizhou, Yunnan), Myanmar, Thailand, Vietnam (Lao Cai (Fansipan), Kon Tum (Ngọc Linh), Dak Lak (Ban 2005); Da Nang (Ba Na)).

Phenology: Flowering from April to May, fruiting in October of following year.

Ecology: Scattered in the pine-forests, evergreen forests.

Vernacular name: Sồi lĩnh.

***Quercus auricoma* A.Camus**, Chênes Atlas 2: 122, pl. 230 (1935); Ho, Ill. Fl. Vietnam 2: 657 (2003); Ban, List Pl. Vietnam 2: 262 (2005); Phengklai, Fl. Thailand 9(3): 361 (2008), nom. tant.

Type: VIETNAM. "Tonkin, massif de Núi Biên près de Chobo"*, Poilane 13098* (P [P00379264, P00379265, P00379263], image!)

Specimens examined: VIETNAM. Hoa Binh Province, Mt. Bien, alt. 1198m, 01 Sept. 19296 *Poilane 13098* (P [P00379263, P00379264, P00379265, image!]); Hue Province, Mt. Bach Ma, alt. 1450m, *Poilane 29625* (VNM [VNM00022815!]); Da Nang Province, Mt. Son Tra, 16°08'02.62"N, 108°14'35.16"E, alt. 518 m, 30 May 2015, *Ngoc Nguyen et al. V3135* [ster.] (FU!, DLU); ibid. 16°08'06.42"N, 108°14'43.53"E, alt. 556 m, 30 May 2015, *Ngoc Nguyen et al. V3138* [ster.] (FU!, DLU).

Distribution: Endemic to Vietnam (Hoa Binh, Quang Tri, Bach Ma - Thua Thien Hue, Son Tra - Da Nang).

Phenology: Flowering from January to May, fruiting from June to September.

Ecology: Lower montane forests, pine-forests, mixed evergreen forests, alt. 518 – 1400m.

Vernacular name: Sồi tóc vàng.

Note: After we examined morphological traits of the type specimen of *Q. auricoma* described from Vietnam, and description of *Quercus* species under name "*Q. auricoma"* in the Flora of Thailand (Phengklai 2008) and our new specimens collected from Vietnam (*V3135, V3138*) and Thailand (*T4287*), we concluded that the species identified as "*Q. auricoma"* in Thailand is not *Q. auricoma* but *Q. cambodiensis* Hickel & A.Camus. *Quercus auricoma* is distinct from *Q. cambodiensis* in that leaves are entire leaves (vs. serrate along the upper 1/2 – 1/3 margin in *Q. cambodiensis*), suborbicular nuts (vs. ovoid to oblong), and cupules sparsely hairy cupules (vs. densely hairy). Consistent with this morphological evidence, MIG-seq tree showed that the species identified as "*Q. auricoma"* in Thailand (*T4287*) is sister to *Q. cambodiensis* (*C4302*) from Cambodia with 100% bootstrap values. *Quercus auricoma* circumscribed here is endemic to central Vietnam.

Quercus austrocochinchinensis **Hickel & Camus**, Ann. Sci. Nat., Bot. 10, 3: 386 (1921); Ho, Ill. Fl. Vietnam 2: 657 (2003); Ban, List Pl. Vietnam 2: 262 (2005); Phengklai, Fl. Thailand 9(3): 363 (2008). —*Cyclobalanopsis austrocochinchinensis* (Hickel & A.Camus) Hjelmq, Dansk Bot. Ark. 23: 503 (1968); Huang et al., Fl. China 4: 397 (1999).

Type: LAOS. Mt. Mu Xoai, alt. 400m, *Pierre 4971* (P [P00379279; P00379280, P00379278, image!]); VIETNAM. North of Vietnam (Tonkin), *Balansa et al. 566* (P00379246, image!].

Specimens examined: VIETNAM. Da Nang Province, Mt. Son Tra, 16°06'58.72"N, 108°14'59.89"E, alt. 249 m, 30 May 2015, *Ngoc Nguyen et al. V3129* [young fr.] (FU!, DLU); ibid. 16°08'22.90"N, 108°15'28.85"E, alt. 340 m, 30 May 2015, *Ngoc Nguyen et al. V3156* [young fr.] (FU!, DLU).

Distribution: This species occurs in Thailand, Laos and China (Yunnan) and Vietnam. In Vietnam, this species has been recorded from Quang Ninh, Vung Tau, Dong Nai (Ban 2005) and Da Nang.

Phenology: Flowering in January, fruiting from February to December.

Ecology: Lower montane forests, on slopes of streams valleys in tropical evergreen forest.

Vernacular name: Sồi Nam Bộ, Sồi cực nam.

Quercus baniensis **A.Camus**, Chênes Atlas 2: 123, pl. 231 (1935-1936), nom. nud.; Bull. Soc. Bot. France 83: 343 (1936); Binh et al. (2018b).

Type: VIETNAM. "Mont Bani, in the main coast range about 25 kilometers from Tourane", 4–13 June 1927, *Clemens 3455* (P [P00753998!], U [U0238780, image!]).

Specimens examined: VIETNAM. Dang Nang Province, Ba Na Nature Reserve, 16°00'07.30"N, 108°01'33.90"E, alt. 707 m, 29 May 2015, *Tagane et al. V3089* [young fr.] (DLU, FU!); ibid., 16°00'10.0"N, 108°02'17.8"E, alt. 789 m, 19 Feb. 2017, *Hoang T.S. & Tagane S. V6922* [ster.] (DLU, FU!).

Distribution: Endemic to Vietnam (Da Nang).

Phenology: Fruiting in June.

Ecology: Edge of evergreen forest, roadside.

Vernacular name: Sồi bà nà.

***Quercus baolamensis* Binh & Ngoc, sp. nov.**; Binh et al. (2018b).

Type: VIETNAM. "Lam Dong Province, Bao Lam District, B40 Pass, 11°43'37"N, 107°42'34.5"E, alt. 1000 m", 13 June 2015, *Ngoc et al. V3191*! [fr.] (FU!, DLU).

Specimens examined: VIETNAM. Lam Dong Province, Bao Lam District, B40 Pass, 11°43'37"N, 107°42'34.5"E, alt. 1000 m, 13 June 2015, *Ngoc et al. V3191* [fr.] (FU!, DLU)

Distribution: Endemic to Vietnam (Bao Lam).

Phenology: Fruiting in June.

Ecology: Edge of evergreen forest, roadside.

Vernacular: Sồi bảo lâm.

***Quercus bella* Chun & Tsiang.**, J. Arnold Arbor. 28: 326 (1947); —*Cyclobalanopsis bella* (Chun & Tsiang) Chun ex Y.C.Hsu & H.W.Jen., J. Beijing Forest. Univ. 15(4): 45 (1993); Huang et al., Fl. China 4: 393 (1999); PhytoKeys 92: 1 (2018).

Type. CHINA. "Fang-Cheng Hsien, Shi-Wan-Ta Shan", alt. 200–250 m, *Chun 4772* (IBSC [catalogue no. 0039624, image!]).

Specimens examined. VIETNAM. Ha Noi Capital, Ba Vi District, Ba Vi National Park, 21°04'40.6"N, 105°22'17.2"E, alt. 600 m, 11 Sep. 2016, *Binh et al. V6044* [ster.]*, V6038* [fr.] (FU!); 21°04'59.6"N, 105°22'03.6"E, alt. 703m, 21 Sep. 2017, *Yahara et. al. V6981* [fr.] (DLU, FU!); 21°03'33.7"N, 105°21'39.4"E, alt. 1023 m, 11 Sep. 2016, *Binh et al. V6031* [fr.] (FU!).

Distribution. This species occurs in China (Guangdong, Guangxi, Hainan) and Vietnam. In Vietnam, this species has been recorded from Mt. Ba Vi, northern Vietnam.

Phenology. Flowering from February to April, fruiting from October to December in China (Huang et al. 1999). Flowering and fruiting specimens were collected from Vietnam in September.
Ecology. In sparsely wooded ravine along stream on moist sites, slopes in evergreen forests.
Vernacular name: Sồi bella.

***Quercus bidoupensis* Binh & Ngoc, sp. nov.**; Binh et al. (2018b).
Type: VIETNAM. "Lam Dong Province: Bidoup-Nui Ba National Park, 12°09'52.95"N, 108°32'00.38"E, alt. 1698 m", 24 Feb. 2016, *Tagane et al. V4328* [ster.] (FU!, DLU).
Specimens examined: VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park, 12°09'52.95"N, 108°32'00.38"E, alt. 1698 m, 24 Feb. 2016, *Tagane et al. V4328* [ster.] (FU!, DLU); ibid. Lan Tranh, 12°04'08.5"N, 108°21'55.5"E, alt. 1695 m, 18 June 2015, *N. Nguyen et al. V3202* [ster.] (DLU, FU!).
Distribution: Endemic to Vietnam (Lam Dong).
Phenology: Unknown.
Ecology: Hill evergreen forest.
Vernacular name: Sồi bi đúp.

***Quercus blakei* Skan**, Hooker's Icon. Pl. 27: t. 2662 (1899); Ho, Ill. Fl. Vietnam 2: 657 (2003); Ban, List Pl. Vietnam 2: 262 (2005). —*Cyclobalanopsis blakei* (Skan) Schottky, Bot. Jahrb. Syst. 47: 649 (1912); Huang et al., Fl. China 4: 393 (1999).
Type: CHINA. Hong Kong, alt. 152 m (500 ft.) *Ford 622* (P [P00721885, image!]; K [K000832100, K000832099, image!]);
Specimens examined: CHINA. Hai Nam, Fan Ya to Yik Tsok Mau mountain, *McClure 9651* (P [P00721886, image!]). VIETNAM. Ha Tinh Province, Vu Quang National Park, alt. 741 m, 18°16'04.4"N, 105°21'10.4"E, 25 July 2016, *Nguyen et al. V 3737* [young fr.] (FU!, DLU).
Distribution: This species occurs in China, Laos and Vietnam. In Vietnam, this species has been recorded from Nghe An (Quy Chau), Lang Son (Bac Son, Vu Le) (Ban 2005, Tran Hop 2002) and Vu Quang.
Phenology: Flowering from May to June, fruiting from December to February.
Ecology: Evergreen tropical forests, hill montane forest.
Vernacular name: Sồi lá mỏng, Sồi Blake, Dẻ lá mỏng.

***Quercus blaoensis* A.Camus**, Chênes Atlas 2: 121, pl. 229 (1935–1936), nom. nud.; Les Chênes 1: 293 (1935); Binh et al. (2018b).

Type: VIETNAM. "Station agricole de Blao, province du haut Donaï", alt. 800 m, 25 Apr 1933, *Poilane* 22372 (P [P00754000], [P00753999!]; K [K000832201, K000832202, K000832203, K000832204]; G [G00358072], image!);

Specimens examined: VIETNAM. Khanh Hoa Province, Hon Ba Nature Reserve, 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 24 Feb. 2014, *Toyama et al. V1366* [young fr.] (FU!, VNM).

Distribution: Endemic to Vietnam (Khanh Hoa).

Phenology: Fruiting in April.

Ecology: Hill evergreen forest.

Vernacular name: Sồi Bờ lao.

***Quercus braianensis* A.Camus**, Chênes Atlas 2: 120 (1935); Ho, Ill. Fl. Vietnam 2: 658 (2003); Ban, List Pl. Vietnam 2: 262 (2005). —*Quercus erioclada* A.Camus, Chênes Atlas 2: 126 (1935).

Type: VIETNAM. "Djiring, alt. 1000–1200 m, *Poilane 24167* (P [P00379261, image!])".

Specimens examined: VIETNAM. Lam Dong Province, Mt. Bi-doup, alt. 2000 m, *Poilane 31012* (P [P00379260, image!]); ibid. Braian Pass, Dankia, Langbiang, alt. 2000 m, *Poilane 18714* (P [P00379262, image!]); ibid. Dalat, alt. 1600 m, *Poilane 30372* [ster.] (VNM [VNM00022861!]); Lam Dong Province, Lan Tranh, 12°08'50.3"N, 108°23'15.4"E, alt. 1535 m, 18 June 2015, *Nguyen et al. V3219* [ster.] (DLU, FU!); Lam Dong Province, Bidoup Nui Ba National Park, 12°10'34.7"N, 108°41'08.4"E, alt. 1533 m, 21 Feb. 2016, *Tagane et al. V4034* [ster.] (DLU, FU!); ibid. 12°09'52.95"N, 108°32'00.38"E, alt. 1698 m, 24 Feb. 2016, *Tagane et al. V4348* [ster.] (DLU, FU); 12°10'17.2"N, 108°41'50.1"E, alt. 1464 m, 26 Feb. 2016, *Tagane et al. V4445* [ster.] (DLU, FU!); Kon Tum Province, Ngoc Linh Nature Reserve, 15°12'32.4"N, 107°43'54.9"E, alt. 1141 m, 10 Feb. 2017, *Tagane et al. V6073* [ster.], *V6077* [ster.] (DLU, FU!); ibid. 15°12'24.2"N, 107°46'10.4"E, alt. 1365 m, 14 Feb. 2017, *Tagane et al. V6633* [ster.] (DLU, FU!).

Distribution: Endemic to Vietnam (Lam Dong, KonTum).

Phenology: Fruiting in October.

Ecology: Lower montane evergreen forest.

Vernacular name: Sồi Braian, Sồi long.

Quercus camusiae **Trel. ex Hickel & A.Camus**, Fl. Indo-Chine [P.H. Lecomte et al.] 5: 957 (1929); Ho, Ill. Fl. Vietnam 2: 658 (2003); Binh et al. (2018b). —*Quercus geminata* Hickel & A.Camus, Bull. Mus. Natl. Hist. Nat. 1923, 29: 599 (1923), nom. illegit. —*Cyclobalanopsis camusiae* (Trel. ex Hickel & A.Camus) Y.C.Hsu & H.W.Jen, J. Beijing Forest. Univ. 15(4): 44 (1993); Huang et al., Fl. China 4: 386 (1999).

Type: VIETNAM. ‘‘Pres de Nha-trang, massif de Honba”, alt. 1,000–1,500 m, 18–20 Sep. 1918, *A. Chevalier 38650* (P [P00379252, P00379253!]);

Specimens examined: VIETNAM. Khanh Hoa Province, Hon Ba Nature Reserve, 12°07'08.64"N, 108°56'51.99"E, alt. 1498 m, 18 July 2013, *Tagane et al. V290* [ster.] (FU!, VNM); 12°07'10.02"N, 108°56'51.71"E, alt. 995 m, 19 July 2013 [young fr.], *Tagane et al. V342* [young fr.] (FU!, VNM); 12°07'11.42"N, 108°57'25.76"E, alt. 1336 m, 25 Nov. 2014, *Toyama et el. V2173* [ster.] (FU!, VNM).

Distribution: China and Vietnam. In Vietnam, this species has been recorded only from the type locality, Nha Trang.

Phenology: Fruiting from July to September.

Ecology: Lower montane evergreen forest.

Vernacular name: Sồi Camus.

Quercus chevalieri **Hickel & Camus**, Ann. Sci. Nat., Bot. sér. 10, 3: 380 (1921); Ho, Ill. Fl. Vietnam 2: 658 (2003); Ban, List Pl. Vietnam 2: 263 (2005). —*Cyclobalanopsis chevalieri* (Hickel & A.Camus) Y.C.Hsu & H.Wei Jen, J. Beijing Forest. Univ. 15(4): 45 (1993); Huang et al., Fl. China 4: 389 (1999).

Type: VIETNAM. “Lao Kay, Chapa, 1400–1500 m”, *Chevalier 29488* (P [P00753900; P00753901; P00753902, image!]).

Specimens examined: VIETNAM. Kon Tum Province, Ngoc Linh Nature Reserve, 15°10'07.4"N, 107°45'43.4"E, alt. 1376 m, 13 Feb. 2017, *Tagane et al. V6448* [ster.] (DLU, FU!].

Distribution: China, Vietnam (Lao Cai, Kon Tum).

Phenology: Fruiting from August to December.

Ecology: Lower montane evergreen forests.

Vernacular name: Sồi Chevalier.

***Quercus chrysocalyx* Hickel & A.Camus**, Ann. Sci. Nat., Bot. sér. 10, 3: 385 (1921); Ho, Ill. Fl. Vietnam 2: 659 (2003); Ban, List Pl. Vietnam 2: 263 (2005).

Type: VIETNAM. "Tien Yen, *Tsang 30687* (E [E00275406], image!)".

Specimens examined: VIETNAM. Ha Tinh Province, Huong Son, alt. 250 m, 17 Aug. 1963, *Loc et al. 2509* (P [P06859101, image!]; ibid. Vu Quang National Park, 18°16'38.4"N, 105°22'18"E, alt. 146 m, 21 July 2015, *Nguyen et al. V3289* [ster.] (DLU, FU!).

Distribution: Laos, Cambodia and Vietnam. In Vietnam, this species has been recorded from Lai Chau, Lao Cai (Sapa), Quang Ninh (Tien Yen) (Ban 2005), Ha Tinh.

Phenology: Fruiting in January.

Ecology: Lowland evergreen forest.

Vernacular name: Sồi quang, Dẻ quang, Sồi đấu vàng.

***Quercus djiringensis* A.Camus,** Chênes Atlas 2: 124 (1935); Ho, Ill. Fl. Vietnam 2: 659 (2003); Ban, List Pl. Vietnam 2: 264 (2005).

Type: VIETNAM. "Donai Province, Braian pass, Djiring", alt. 1000–1200 m, 1 Jan. 1935, *Poilane* (P [P00379250, P00379251, image!]);

Specimens examined: VIETNAM. Donai Province, *Poilane 24771* [ster.] (VNM [VNM00022829!]); Lam Dong Province, Bidoup Nui Ba National Park, 12°09'32.53"N, 108°32'09.55"E, alt. 1633 m, 24 Feb. 2016, *Tagane et al. V 4309* [ster.] (DLU, FU!); ibid. *Dung et al. V5537* [ster.], *V5538* [ster.] (DLU, FU!).

Distribution: Endemic to Vietnam (Dong Nai and Lam Dong).

Phenology: Flowering from September to December.

Ecology: Hill evergreen forests.

Vernacular name: Sồi di linh.

***Quercus disciformis* Chun & Tsiang.**, J. Arnold Arbor. 28: 324 (1947); PhytoKeys 92, 1 (2018).

Cyclobalanopsis disciformis (Chun & Tsiang) Y.C.Hsu & H.W. Jen, Acta Bot. Yunnan. 1: 148 (1979); Huang et al., Fl. China 4: 397 (1999).

Type: CHINA. "Hsin-I Hsien, Ling-Tung Pao, Chung-Tung", 3 Aug. 1931, *C. Wang 31087* (IBK [IBK00081941], IBSC [IBSC0117316, image!]);

Specimens examined: VIETNAM. Ha Noi Capital, Ba Vi National Park, alt. 737 m, 21°04'33.88"N, 105°22'03"E, 12 Sept. 2016, *Binh et al. V6052* [ster.]*, V6053* [ster.]*, V6058* [fr.] (FU!); 21°03'34.1"N, 105°21'54.1"E, alt. 1172 m, 11 Sep. 2016, *Binh et al. V6040* [fr.] (FU!).
Distribution. China (Guangdong, Guangxi, Guizhou, Hainan, Hunan), Vietnam (Ba Vi National Park).
Phenology: Flowering from March to April, fruiting from August to September.
Ecology: Broad-leaved evergreen forests in mountains.
Vernacular name: Sồi.

***Quercus donnaiensis* A.Camus**, Chênes Atlas 2: 119, pl. 227 (1935–1936), nom. nud.; Les Chênes 1: 190 (1935). Binh et al. (2018b).
Type: VIETNAM. "Annam: Près de Sapoum, près station agricole de Blao, prov. du Haut Donai", alt. 1000–1100 m, 9 Jan. 1935, *Poilanei 23732* (P [P00753995, P00753994!]).
Specimens examined: VIETNAM. Lam Dong Province, Lan Tranh wards, 12°04'08.5"N, 108°21'55.5"E, alt. 1695 m, 18 June 2015, *N. Nguyen et al. V3208* [ster.] (DLU, FU); Lam Dong Province, Bi Doup-Nui Ba National Park, 12°11'19.8"N, 108°40'48.3"E, alt. 1489 m, 25 Feb. 2016, *Tagane et al. V4398* [young fr.] (DLU, FU!).
Distribution: Endemic to Vietnam (Lam Dong).
Phenology: Fruiting in January.
Ecology: Lower montane evergreen forest, beside a stream and edge of evergreen forest.
Vernacular name: Sồi đồng nai.

***Quercus helferiana* A. DC.**, Prodr. [A. P. de Candolle] 16(2.1): 101 (1864); Ho, Ill. Fl. Vietnam 2: 661 (2003); Ban, List Pl. Vietnam 2: 266 (2005); Phengklai, Fl. Thailand 9 (3): 371 (2008). — *Cyclobalanopsis helferiana* (A.DC.) Oerst., Vidensk. Meddel. Naturhist. Foren. Kjøbenhavn 18: 79 (1866); Huang et al., Fl. China 4: 391 (1999).
Type: INDIA. (n.v.).
Specimens examined: VIETNAM. Phan Rang Province, Ca Na, alt. 700 – 800m, *Poilane 12315* [fr.] (VNM [VMN00020148, image!]); Lam Dong Province, Bidoup Nui Ba National Park, 19 June 2015, *Ngoc et al. V3244* [young fr.] (DLU, FU!); ibid. Mimosa Pass, 11°54'59.8"N, 108°27'27.8"E, alt. 1400 m, 13 June 2015, *Nguyen et al. V3169* [young fr.] (DLU, FU!).

Distribution: India, Laos, Thailand, China, Myanmar and Vietnam. In Vietnam, this species has been recorded from Son La, Khanh Hoa, Kon Tum, Ninh Thuan, Lam Dong.
Phenology: Flowering from March to April, fruiting from July to October.
Ecology: Edge of evergreen forest, roadside.
Vernacular name: Sồi quả dẹt, Dẻ gạo, Sồi Helfer.

***Quercus honbaensis* Binh, Tagane & Yahara, sp. nov**. Binh et al. (2018b).
Type: VIETNAM. "Khanh Hoa Province, Hon Ba Nature Reserve, 12°07'22.79"N, 109°00'13.29"E, alt. 225 m", 24 Feb. 2014, *Toyama et al. V1378* [fr.] (DLY, FU!).
Specimens examined: VIETNAM. Khanh Hoa Province, Hon Ba Nature Reserve, 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 24 Feb. 2014, *Toyama et al. V1378* [fr.] (DLU, FU); ibid. 12°06'33.41"N, 108°59'24.89"E, alt. 367 m, 22 July 2013, *Tagane et al. V744* [ster.] (DLU, FU!); 12°06'39.77"N, 108°58'59.23"E, alt. 617 m, 22 Feb. 2014, *Toyama et al. V1200* [ster.] (DLU, FU!); 12°06'31.2"N, 108°59'14.1"E, alt. 400 m, 13 July 2014, *Tagane et al. V1548* [ster.] (DLU, FU!); 12°07'22.79"N, 109°00'13.29"E, alt. 225 m, 15 July 2014, *Tagane et al. V1662* [ster.] (DLU, FU!).
Distribution: Endemic to Vietnam (Hon Ba).
Phenology: Fruiting in February.
Ecology: Evergreen forests.
Vernacular name: Sồi hòn bà.

***Quercus kerrii* Craib.**, Bull. Misc. Inform. Kew 1911: 471 (1911); Ho, Ill. Fl. Vietnam 2: 661 (2003); Ban, List Pl. Vietnam 2: 266 (2005)*;* Phengklai, Fl. Thailand 9 (3): 373 (2008). — *Cyclobalanopsis kerrii* (Craib) Hu, Bull. Fan Mem. Inst. Biol. 10: 106 (1940). Huang et al., Fl. China 4: 391 (1999).
Type: THAILAND. "Doi Sootep, Chiang Mai", alt. 300–600 m, 14 March 1909, *Kerr 550* (K [K000227635, image!]); ibid. alt. 1,000 m, 2 Feb. 1905, *Hosseus 438* (M [M0168772, image!]);
Specimens examined: VIETNAM. Kon Tum Province, Ngoc Linh Nature Reserve, 15°11'15.7"N, 107°50'27.2"E, alt. 811 m, 16 Feb. 2017, *Tagane et al. V6765* [ster.] (DLU, FU!); ibid. 15°12'24.2"N, 107°46'10.4"E, alt. 1365 m, 14 Feb. 2017, *Tagane et al. V6677* [fl.] (DLU, FU!). CAMBODIA. Mondulkiri Province, Mt. Sen Monorom, 12°30'24.62"N, 107°16'40.36"E, alt. 638

m, 03 Nov. 2016, *Tagane* et al. *C7223* [fr.] (FU!, the herbarium of Forest Administration of Cambodia).

Distribution: China (Guangxi, Guizhou, Hainan, Yunnan), Thailand (Mae Hong Son, Chiang Mai, Chiang Rai, Lampang, Phrae, Uttaradit, Tak, Phetchabun, Loei, Chaiyaphum, Kanchanaburi, Phetchaburi, Ranong). In Vietnam, this species has been recorded from Son La, Kon Tum, Gia Lai, Lam Dong, Ninh Thuan (Ban 2005).

Phonology: Flowering from March to April, fruiting from January to October.

Ecology: On slopes of stream valley in tropical evergreen forests, mixed deciduous forests, rain tropical forests, hill evergreen forests.

Vernacular name: Sồi gân phẳng, Sồi Kerr, Sồi lá nhót.

***Quercus lanata* Smith.**, Cycl. 29: 27 (1819); Ho, Ill. Fl. Vietnam 2: 662 (2003); Ban, List Pl. Vietnam 2: 267 (2005)*;* Phengklai, Fl. Thailand 9 (3): 379 (2008)*;* Huang et al., Fl. China 4: 379 (1999).

Type: NEPAL. (n.v.).

Specimens examined: VIETNAM. "Annam: Nord de Dran et Ka-Nam pro: du Haut Donaï", 11°54'58.068"N; 108°38'18.24"E, alt. 1200 m, *Poilane 30566* (P [P06872388, image!]); "Annam: Sud du Bi-Doup pro: du Haut Donaï", 12°4'59.988"N; 108°40'0.012"E, alt. 1500 m, *Poilane 30677* (P [P06872387, image!]); Lam Dong Province, Bidoup Nui Ba National Park, 19 June 2015, *Nguyen et al. V3228* [fl., young fr.] (DLU, FU!).

Distribution: China (Guangxi, Xizang, Yunnan), Thailand (Chiang Mai) and Vietnam. In Vietnam, this species has been recorded from Lam Dong.

Phenology: Flowering from June to August, fruiting from January to February in the next year.

Ecology: Lower montane forests, edge of evergreen forest.

Vernacular name: Sồi (cau) lông, Sồi trái nhẵn.

***Quercus langbianensis* Hickel & A.Camus**, Ann. Sci. Nat., Bot. 10, 3: 382 (1921); [P.H. Lecomte et al.] Fl. Indo-Chine [P.H. Lecomte et al.] 5: 950 (1929); Ho, Ill. Fl. Vietnam 2: 662 (2003); Ban, List Pl. Vietnam 2: 267 (2005); Binh et al. (2018b).

Type: VIETNAM. "Annam: massif du Lang-Bian, grand Piton Lang-Bian, près du village de Beneur", alt. 1500–2000 m, 15 Feb. 1914, *Chevalier 30029* (P [P00379254, P00379255, P00379256, image!]);

Specimen examined: VIETNAM. Lam Dong Province, Bidoup-Nui Ba National Park, 12°10'34.04"N, 108°40'28.93"E, alt. 1472 m, 29 Feb. 2016, *Tagane et al. V4465* [fr.] (DLU, FU!)*;* ibid. 12°10'34.09"N, 108°41'04"E, alt. 1533 m, 22 Feb. 2016, *Tagane et al. V4165* [ster.], *V4166* [fr.] (DLU, FU!)*;* 12°10'34.7"N, 108°41'08.4"E, alt. 1533 m, 21 Feb. 2016, *Tagane et al. V3962* [ster.] (DLU, FU!).

Distribution: Endemic to Vietnam (Mt. Langbian, Lam Dong).

Phenology: Fruiting in February.

Ecology: Lower montane evergreen forest.

Vernacular name: Sồi guồi, sồi lang bian.

***Quercus macrocalyx* Hickel & A.Camus**, Ann. Sci. Nat., Bot. 10, 3: 383 (1921); Ho, Ill. Fl. Vietnam 2: 663 (2003); Ban, List Pl. Vietnam 2: 268 (2005). —*Cyclobalanopsis fleuryi* (Hickel & A.Camus) W.T. Chun, in Fl. Fujianica 1: 403 (1982); Huang et al., Fl. China 4: 385 (1999). —*Cyclobalanopsis macrocalyx* (Hickel & A.Camus) M. Deng & Z.K. Zhou, Ann. Bot. Fenn. 43: 57 (2006); —*Quercus fleuryi* Hickel & A.Camus, Bull. Mus. Natl. Hist. Nat. 29: 600 (1923). —*Quercus megalocarpa* A.Camus, Chênes Atlas 3: 50 (1948). ─*Cyclobalanopsis macrocalyx* (Hickel & A.Camus) M. Deng & Z.K. Zhou, Ann. Bot. Fenn. 43: 57 (2006).

Type: LAOS. "Haut cours de la Tchépone, entre Lang Ka va Sam et Lang A Rum", 16°15'0"N, 107°1'1.2"E, alt. 1000 m, 26 July 1925, *Poilane 12208* (P [P06813035, image!]).

Specimens examined: VIETNAM. Ha Noi Capital, Ba Vi mountain, Son Tay, alt. 800 – 1200 m, 2 June 1918, *Fleury 37831* (P [P00753925, image!]); Hue Province, Bach Ma National Park, 16°11'56.87"N, 107°51'26.01"E, alt. 1412 m, 27 May 2015, *Yahara et al. V2994* [ster.] (DLU, FU!); ibid. 16°11'49.05"N, 107°51'22.43"E, alt. 1348 m, 27 May 2015, *Yahara et al. V3005* [ster.] (DLU, FU!); Ha Tinh Province, Vu Quang National Park, 18°11'34.9"N, 105°23'08.9"E, alt. 1634 m, 22 June 2016, *Yahara et al. V5776* [fr.] (DLU, FU!); ibid. 18°12'20.0"N, 105°23'23.3"E, alt. 1335 m, 24 June 2016, *Yahara et al. V5928* [ster.] (DLU, FU!); Kon Tum Province, Ngoc Linh Nature Reserve, 15°10'07.4"N, 107°45'43.4"E, alt. 1376 m, 13 Feb. 2017, *Tagane et al. V6457* [fr.] (DLU, FU!).

Distribution: China (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Jiangxi, Xizang, Yunnan), Laos and Vietnam. In Vietnam, this species has been recorded from Thai Nguyen, Vinh Phuc, Thua Thien Hue, Da Nang, Gia Lai, Kon Tum, Son La (Ban, 2005), Ha Tinh.
Phenology: Fruiting from May to December.
Ecology: Lower montane evergreen forests.
Vernacular name: Sồi đấu to, Sồi nón cụt.

***Quercus neglecta* (Schottky) Koidz.**, Bot. Mag. (Tokyo) 30: 201 (1916). —*Cyclobalanopsis neglecta* Schottky, Bot. Jahrb. Syst. 47: 650 (1912); Huang et al., Fl. China 4: 387 (1999). —*Quercus bambusifolia* Hance, Bot. Voy. Herald 415 (1857), nom. nud., J. Bot. (London) 13: 364, later homonym; Ho, Ill. Fl. Vietnam 2: 657 (2003); Ban, List Pl. Vietnam 2: 262 (2005).
Type: China. "Hong Kong", (P [P06779971, image!], PE [PE01605284, image!]).
Specimens examined: VIETNAM. Hue province, Mt. Bach Ma, 16°11'51.72"N; 107°51'37.83"E, alt. 1400–1500 m, 6 September 1938, *Poilane 27646* (P [P06779947, image!]); Ha Tinh Province, Vu Quang National Park, 18°16'10.3"N; 105°21'30"E, alt. 546 m, 24 July 2015, *Nguyen et al. V3587* [fl., fr.] (DLU, FU!); ibid. 18°15'40"N; 105°20'50"E, alt. 1062 m, 26 July 2015, *Nguyen et al. V3788* [fr.], *V3791* [ster.] (DLU, FU!).
Distribution: China (Guangdong, Guangxi, Hainan) and Vietnam. In Vietnam, this species has been recorded from Yen Bai, Tuyen Quang, Quang Ninh, Thanh Hoa, Nghe An, Quang Binh, Thua Thien Hue, Ha Tinh (Ban 2005).
Phenoloygy: Flowering from February to March, fruiting from July to November.
Ecology: Evergreen hill forests.
Vernacular name: Sồi lá tre, Sồi tre.

***Quercus platycalyx* Hickel & A.Camus**, Ann. Sci. Nat., Bot. 10, 3: 380 (1921); Ban, List Pl. Vietnam 2: 269 (2005).
Type: VIETNAM. (n.v.)
Specimens examined: VIETNAM. Ha Tinh Province, Vu Quang National Park, 15 Oct. 2015, *Nguyen et al. V6065* [fr.] (FU!).
Distribution: Endemic to Vietnam (Cao Bang, Lang Son, Tuyen Quang, Phu Tho, Quang Ninh, Vinh Phuc, Ha Tay, Hoa Binh, Nghe An, Ha Tinh).

Phenology: Flowering from June to August, fruiting from December to February

Ecology: Secondary forests on clay soil.

Vernacular name: Sồi đĩa, Mạy có.

Note: Ho (2003) treated this species as a synonym of *Q. quangtriensis*. However, we collected both *Q. platycalyx* and *Q. quangtriensis* in Vu Quang National Park, at 40 m and 650 m, respectively, and the two species are distinct in leaf morphology; leaves almost elliptic-lanceolate, margin slightly serrate in upper 1/2, apex acute, tertiary veins abaxially faint in *Q. platycalyx* (vs. leaves lanceolate or elliptic-lanceolate, margin serrate in upper 1/2, apex acuminate, tertiary veins abaxially conspicuous in *Q. quangtriensis*). The specimen identified as *Q. platycalyx* (V6065) is considered to be identical with the drawing of Camus (1934) in having unique morphology of obovoid nuts narrowed at base and placed on a shallow discoid cupule, while the type specimen of *Q. quangtriensis* has an ovoid nut enclosed by a cup-shaped cupule. Our collection of *Q. quangtriensis* (V3578) has nuts similar to the type specimen of *Q. quantriensis*. In the original publication of *Quercus platycalyx* (Hickel and Camus 1921), the specimen collected by Balansa from "Tonkin, vallée de Lankok, mont Bavi; at alt. 400 mètres" were cited but lacking any collection numbers. Later, Camus (1934) illustrated mature fruit and cupule of this species in Chênes Atlas vol. 1 (Pl. 2). However, we cannot find any specimens of Balansa in HN, VNM, P, K, and PE herbaria. In our field surveys, we collected 14 specimens of *Quercus* from Vu Quang National Park, among which a specimen (*V6065* [fr.]) is consistent with the original description and illustration of *Q. platycalyx* of Hickel and Camus (1921) and Camus (1934). Besides, we also collected four specimens of *Quercus disciformis* from Ba Vi National Park (*V6052, V6058, V6040, V6053*) where *Q. platycalyx* was originally collected by Balansa. However, comparison based on morphological characters all of these specimens in the field and the molecular evidence for specimens from Ba Vi National Park and *Q. platycalyx* (*V6065*) from Vu Quang National Park revealed that they are different species. About morphological traits, *Q. platycalyx* is distinguished from *Q. disciformis* in having dish–shaped cupule which is 0.8 cm high and 2.8 cm in diam. (vs. discoid cupule, rim flat, 2.5 – 3 cm in diam.), entire margin of cupule rings (vs. denticulate except apical 2 or 3 rings which is entire) obovoid nut, 3.5 cm high, 1.7 cm in diam. (vs. oblate nut, 1.8 – 2 cm high, 2.1 – 2.3 cm in diam.). Our molecular evidence showed that *Q. disciformis* was sister to *Q. bella* and separated from the *Q. platycalyx* with high supports in MIG-seq tree (bootstrap probability 91%).

***Quercus poilanei* Hickel & A.Camus**, Ann. Sci. Nat., Bot. 10, 3: 384 (1921); Ho, Ill. Fl. Vietnam 2: 664 (2003); Ban, List Pl. Vietnam 2: 269 (2005); Phengklai, Fl. Thailand 9 (3): 393 (2008). — *Cyclobalanopsis poilanei* (Hickel & A.Camus) Hjelmq., Dansk Bot. Arkiv. 23: 508 (1968); Huang et al., Fl. China 4:399 (1999).

Type: VIETNAM. "Annam: Bana", 7 June 1920, *E. Poilane 1547* (P [P00753980, P00753981, image!])

Specimens examined: VIETNAM. Da Nang province, Ba Na Nature Reserve, 15°59'57.88"N; 107°59'41.98"E, alt. 1310 m, 29 May 2015, *Tagane et al. V3113* [ster.] (DLU, FU!); Thua Thien Hue Province, Bach Ma National Park, 16°11'41.65"N; 107°51'14.71"E, alt. 1,308 m, 26 May 2015, *Yahara et al. V2907* [ster.] (DLU, FU!); ibid. 16°11'56.87"N; 107°51'26.01"E, alt. 1,=412 m, 27 May 2015, *Yahara et al. V2986* [ster.] (DLU, FU!); Khanh Hoa Province, Hon Ba Nature Resever, 12°07'10.02"N; 108°56'51.71"E, alt. 1511 m, 19 July 2013, *Tagane et al. V339* [ster.] (DLU, FU!); ibid. 12°07'29.45"N; 108°57'51.11"E, alt. 1204 m, 22 July 2013, *Tagane et al. V703* [ster.] (DLU, FU!); 12°07'18.4"N; 108°58'15.9"E, alt. 1021 m, 23 Nov. 2014, *Toyama et al. V1990* [ster.], *V2043* [ster.] (DLU, FU!); Lam Dong Province, Bidoup Nui Ba National Park, 12°11'12.49"N; 108°42'52.16"E, alt. 1644 m, 19 Nov. 2014, *Toyama et al. V1895* [ster.], (DLU, FU!); ibid. 12°11'28.2"N; 108°42'46.8"E, alt. 1807 m, 23 Feb. 2016, *Tagane et al. V4178* [ster.] (DLU, FU!); 19 June 2015, *Ngoc et al. V3224* [ster.] (DLU, FU!).

Distribution: China (Guangxi), Thailand (Mae Hong Son, Chiang Mai), Laos and Vietnam. In Vietnam, this species has been recorded from Lao Cai, Thua Thien Hue, Da Nang, Khanh Hoa, Dak Lak, Lam Dong (Ban, 2005).

Phenology: Flowering from April to June, fruiting starting in October.

Ecology: Oak-pine forest and evergreen forests.

Vernacular name: Sồi bộp, Sồi áo tơi, Dẻ bộp, Sồi poilane.

***Quercus quangtriensis* Hickel & A.Camus**, Bull. Mus. Natl. Hist. Nat. 32: 400 (1926); Ho, Ill. Fl. Vietnam 2: 664 (2003), excluding the synonym of *Quercus platycalyx*; Ban, List Pl. Vietnam 2: 269 (2005); Phengklai, Fl. Thailand 9 (3): 393 (2008).

Type: VIETNAM. Quang Tri Province, "Massif de Dông eo pat", alt. 650 m, 3 July 1924, *Poilane 11118* (P [P00753971, P00753972, image!]; A [A00034183, image!]).

Specimens examined: VIETNAM. Ha Tinh Province, Vu Quang National Park, 18°16'22"N; 105°21'39.3"E, alt. 453 m, 24 July 2015, *Nguyen et al. V3578* [fr.] (DLU, FU!).

Distribution: Endemic to Vietnam (Quang Tri, Quang Nam, Lam Dong, Vu Quang).

Phenology: Flowering from February to April, fruiting from February to November.

Ecology: Lower montane forest and evergreen hill forest.

Vernacular name: Sồi quảng trị.

Note: Flora of Thailand (Phengklai 2008) treated *Q. wangsaiensis* Barnett and *Q. longistyla* Barnett as synonyms of *Q. quangtriensis* Hickel & Camus and this treatment is also found in The Plant List (2013). However, we concluded that *Q. quangtriensis* is distinguished from both *Q. wangsaiensis* and *Q. longistyla* after we carefully compared their morphological traits based on the type specimen of *Q. quangtriensis* (A [A00034183], image!), *Q. wangsaiensis* (BM [BM000839276, image!]), and *Q. longistyla* (BM [BM000839290, image!]). *Quercus quangtriensis* is more similar to *Q. longistyla* in having leaves lanceolate or elliptic-lanceolate, base acute, apex acuminate but differs in having less hairy cupule with almost entire rings of bracts on the cupule (vs. densely hairy cupule with denticulate rings); nuts ovoid and acute at apex, less hairy around stylopodia (vs. nuts ovoid and rounded at apex, densely hairy around stylopodia). *Quercus quangtriensis* distinguished from *Q. wangsaiensis* in having leaves margin serrate in upper 1/2 (vs. leave margin slightly undulate and serrate in upper 1/5 in *Q. wangsaiensis*); nut ovoid (vs. nut ellipsoid). In our field surveys, we collected *Q. longistyla* from the type locality (Phu Kradueng, Thailand; *T4634*) and *Q. quangtriensis* from Vietnam (*V3578*). Molecular evidence from those samples shows that *Q. quangtriensis* was sister to *Q. platycalyx* and not close to *Q. longistyla*.

***Quercus sessilifolia* Blume**, Mus. Bot. 1: 305 (1851). —*Quercus nubium* Hand-Mazz., Anz. Akad. Wiss. Wien, Math. -Naturwiss. Kl. 59: 137 (1922); Ban, List Pl. Vietnam 2: 268 (2005). — *Cyclobalanopsis sessilifolia* (Blume) Schottky, Bot. Jahrb. Syst. 47: 652 (1912). — *Cyclobalanopsis nubium* (Hand. -Mazz.) Chun, Fl. Fujianica 1: 410 (1982); Huang et al., Fl. China 4: 389 (1999).

Type: JAPAN. "Japonia", *Siebold s.n.* (L [L0040844, image!]).

Specimens examined: China, Zhejiang Province, 17 April 1959, Longquan, *Zhang Shaoyao 4759* (AU [AU031844, image!]); Hunan Province, Nanyue district, Nanyue Nature Resever, 6

September 2003, *Li et al. 1110* (PE [PE01359999, image!]). VIETNAM. Lao Cai Province, Hoang Lien National Park, Fansipan mountain, 22°19'48.0"N; 103°46'57.5"E, alt. 2225 m, 2-3 May 2016, *Binh et al. V5047* [ster.], *V5112* [ster.] (DLU, FU!).

Distribution: China (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, Taiwan, Zhejiang), Japan, Taiwan (Atunshan, Chihsingshan, Wuchihshan, Peichatienshan, Lalashan, Talu Forest Road, Kutzulunshan, Tahanshan, Taipingshan) and Vietnam. In Vietnam, this species has been recorded from Quang Ninh (Mong Cai) (Ban 2005), Lao Cai (Mt. Fansipan).

Phenology: Flowering from April to May, fruiting from October to November.

Ecology: Scattered in lower montane evergreen forests.

Vernacular name: Sồi cực nhỏ.

Quercus setulosa **Hickel & A.Camus**, Bull. Mus. Natl. Hist. Nat. 29: 598 (1923); Ho, Ill. Fl. Vietnam 2: 665 (2003); Ban, List Pl. Vietnam 2: 269 (2005); Huang et al., Fl. China 4: 380 (1999); Phengklai, Fl. Thailand 9 (3): 403 (2008). —*Quercus laotica* A.Camus, Chênes Atlas 2: 7 (1935).

Type: VIETNAM. "Annam: Dran, prov. de Lang Biang", alt. 1000 m, 20 June 1922, *Poilane 3891* (P [P00753965, P00753964, image!]).

Specimens examined: VIETNAM. Lam Dong Province, Duc Trong, Lang Hanh, 11°37'44.3"N; 108°16'30.1"E, alt. 890 m, 13 June 2015, *Nguyen et al. V3172* [ster.] (DLU, FU!).

Distribution: Laos (Boloven), Thailand (Phetchabun, Loei, Chaiyaphum), China (Guangdong, Guangxi, Guizhou, Yunnan) and Vietnam. In Vietnam, this species has been recorded from Kon Tum, Gia Lai, Dak Lak (Ban 2005), Lam Dong.

Phenology: Flowering from January to May, fruiting from January to November.

Ecology: Edge of evergreen forest and oak forest on Feralit soil.

Vernacular name: Sồi duối, Sồi lào, Sồi cung.

Quercus songtavanensis **A.Camus**, Les Chenes (Encycl. Econ. Sylv. 8) 3(2): 51 (1948); Ho, Ill. Fl. Vietnam 2: 663 (2003).

Type: VIETNAM. "Tonkin: Massif du Sang ta Van", alt. 1700 m, Aug. 1933, *Pételot 4846* (P [P00753990, P00753989, image!]);

Specimens examined: VIETNAM. Ninh Binh Province, Cuc Phuong National Park, alt. 654 m, Oct. 2016, *Huong et al. V6028* [young fr.] (FU!).

Distribution: Endemis to Vietnam (Lao Cai, Ninh Binh).

Phenology: Fruiting from August to October.

Ecology: Limestone evergreen mixed forest.

Vernacular name: Sồi răng nhọn, Sồi Sangtavan.

***Quercus trungkhanhensis* Binh & Ngoc, sp. nov.**, Acta Phytotax. Geobot., 2018c.

Type: VIETNAM. "Cao Bang Province, Trung Khanh district, Cao Vit Gibbon Conservation Area, 22°54'55"N, 106°31'28"E, alt. 767 m", 6 Nov. 2016, *Binh et al. V6066* [fr.] (DLU, FU!, KYO).

Specimens examined: VIETNAM. Cao Bang Province, Trung Khanh district, Cao Vit Gibbon Conservation Area, 22°54'55"N, 106°31'28"E, alt. 767 m, 6 Nov. 2016, *Binh et al. V6066* [fr.] (DLU, FU!, KYO).

Distribution: Endemic to Vietnam (Cao Bang).

Phenology: Fruiting in January.

Ecology: Limestone subtropical evergreen mixed forest.

Vernacular name: Sồi trùng khánh.

***Quercus xuanlienensis* Binh, Ngoc & Bon, sp. nov.**, PhytoKeys 92: 1 (2018).

Type: VIETNAM. "Thanh Hoa Province, Thuong Xuan District, Xuan Lien Nature Reserve, 19°52'46.7"N, 105°11'34.4"E, alt. 810 m", 6 Mar. 2017, *Binh et al. V6967* [fr.] (holotype KYO; isotpyles FU!; DLU!).

Specimens examined: Thanh Hoa Province, Thuong Xuan District, Xuan Lien Nature Reserve, 19°52'46.7"N, 105°11'34.4"E, alt. 810 m, 6 Mar. 2017, *Binh et al. V6967* [fr.] (holotype KYO; isotpyles FU!; DLU!).

Distribution: Endemic to Vietnam (Thanh Hoa).

Phenology: Fruiting in March.

Ecology: Evergreen forest.

Vernacular name: Sồi xuân liên.

***Quercus xanthoclada* Drake.**, J. Bot. (Morot) 4: 149 (1890); Ban, List Pl. Vietnam 2: 270 (2005). — *Cyclobalanopsis xanthoclada* (Drake) Schottky, Bot. Jahrb. Syst. 47: 650 (1912).

Type: VIETNAM. "Tonkin: Vallée de Lankok (Mont Bavi), dans les forêts", Aug. 1887, *Balansa 2378* (P [P00753951, P00753949], image!).

Specimens examined: VIETNAM. Ha Tinh Province, Vu Quang National Park, 18°16'22"N, 105°21'39.3"E, alt. 453 m, 24 July 2015, *Nguyen et al. V3581* [fr.] (DLU, FU!); ibid., 18°16'09.1"N, 105°21'17.5"E, alt. 649 m, 25 July 2015, *Nguyen et al. V3718* (DLU, FU!).

Distribution: Laos and Vietnam. In Vietnam, this species has been recorded from Lang Son, Bac Can, Thai Nguyen, Ha Tay, Hoa Binh (Ban 2005), Ha Tinh.

Phenology: Fruiting from July to October.

Ecology: Evergreen hill forests.

Vernacular name: Sồi tày, Sồi trụ, Sồi biến, Mai kho.

Undescribed species of *Quercus* in Vietnam recognized in this study

***Quercus pseudocamusiae**, ined.*

Specimens examined: VIETNAM. Ha Tinh Province, Vu Quang National Park, 18°16'30.9"N, 105°21'41.8"E, alt. 407 m, 24 July 2015, *Nguyen et al. V3572* [ster.] (DLU, FU!).

Distribution: Ha Tinh (Vu Quang).

Phenology: Unknown.

Ecology: Hill evergreen forest.

***Quercus fansipanensis**, ined*

Specimens examined: VIETNAM. Lao Cai Province, Hoang Lien National Park, Fansipan mountain, 22°19'48.0"N, 103°46'57.5"E, alt. 2225 m, 03 May 2016, *Binh et al.* V5101 [young fr.] (DLU, FU!).

Distribution: Lao Cai (Fansipan).

Phenology: Fruiting start from May.

Ecology: In lower montane evergreen forest.

***Quercus haivanensis**, ined*

Specimens examined: VIETNAM. Da Nang Province, Hai Van Pass, 16°11'12.85"N, 108°07'25.48"E, alt. 663 m, 28 May 2015, *Toyama et al. V3042* [ster.] (DLU, FU!).

Distribution: Da Nang (Hai Van Pass).

Phenology: Unknown.

Ecology: Evergreen forest, on the trail to intake point.

***Quercus ngoclinhensis**, ined*

Specimens examined: Kon Tum Province, Ngoc Linh Nature Reserve, 15°10'05.7"N, 107°45'23.6"E, alt. 1067 m, 11 Feb. 2017, *Tagane et al. V6136* [ster.] (DLU, FU!).

Distribution: Kon Tum (Ngoc Linh Nature Reserve).

Phenology: Unknown.

Ecology: Lower montane evergreen forest.

***Quercus semiundulata**, ined*

Specimens examined: Kon Tum Province, Ngoc Linh Nature Reserve, 15°11'55.7"N, 107°46'14.3"E, alt. 1354 m, 14 Feb. 2017, *Tagane et al. V6597* [ster.] (DLU, FU!).

Distribution: Kon Tum (Ngoc Linh Nature Reserve).

Phenology: Unknown.

Ecology: Lower montane evergreen forest.

***Quercus sontraensis**, ined*

Specimens examined: Da Nang Province, Son Tra Conservation areas, alt. 200–300 m, 10 Oct. 2016, *Tagane et al. V6965* [fr.] (DLU, FU!).

Distribution: Da Nang (Son Tra).

Phenology: Fruiting in October.

Ecology: Evergreen forest.

***Quercus theifolia**, ined*

Specimens examined: VIETNAM. Kon Tum Province, Ngoc Linh Nature Reserve, 15°11'55.7"N, 107°46'14.3"E, alt. 1354 m, 14 Feb. 2017, *Tagane et al. V6618* [ster.] (DLU, FU!).

Distribution: Kon Tum (Ngoc Linh Nature Reserve).

Phenology: Unknown.

Ecology: Lower montane evergreen forest.

***Quercus tiepii**, ined*

Specimens examined: Lam Dong Province, Bidoup Nui Ba National Park, 12°11'28.2"N, 108°42'46.8"E, alt. 1807 m, 23 Feb. 2016, *Tagane et al. V6597* [ster.] (DLU, FU!).

Distribution: Lam Dong (Bidoup-Nui Ba National Park).

Phenology: Unknown.

Ecology: Upper montane evergreen forest.

***Quercus verticillata**, ined*

Specimens examined: VIETNAM. Lam Dong Province, Bidoup Nui Ba National Park, 12°09'52.95"N, 108°32'00.38"E, alt. 1698 m, 24 Feb. 2016, *Tagane et al. V4365* [ster.] (DLU, FU!).

Distribution: Lam Dong (Bidoup-Nui Ba National Park).

Phenology: Unknown.

Ecology: Montane forest.

***Quercus vuquangensis**, ined*

Specimens examined: Ha Tinh Province, Vu Quang National Park, 18°12'20.0"N, 105°23'23.3"E, alt. 1335 m, 21 June 2016, *Yahara et al. V5724* [ster.], V5927 [ster.] (DLU, FU!).

Distribution: Vietnam (Ha Tinh (Vu Quang National Park)).

Phenology: Unknown.

Ecology: Lower montane forest.

Species recorded from Vietnam but not collected in this study

***Quercus acutissima* Carruth.**, J. Linn. Soc., Bot. 6: 33 (1862); Ho, Ill. Fl. Vietnam 2: 656 (2003); Ban, List Pl. Vietnam 2: 261 (2005). Huang et al., Fl. China 4: 372 (1999); Phengklai, Fl. Thailand 9 (3): 357 (2008).

Type: China. (n.v).

Specimens examined: LAOS. Paksong Province, alt. 1200 m, *Poilane 15671* (VNM [VNM00022834]). VIETNAM. Ha Giang Province, Hoang Su Phi, *s. coll. 7036* [fr.] (HN [HN0000030007!]).

Distribution: Nepal, Laos, India, Japan, Myanmar, China (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Liaoning, Shaanxi, Shandong, Shanxi, Sichuan, SE Xizang, Yunnan, Zhejiang), Thailand (Chiang Mai, Phetchabun, Loei, Chaiyaphum), Korea, U.S.A and Vietnam. In Vietnam, this species has been recorded from Lai Chau, Lao Cai, Son La, Yen Bai (Ban 2005, Tran Hop 2002).

Phenology: Flowering from January to March, fruiting from January to November.

Ecology: Evergreen forest, alt. 900–2200 m.

Vernacular: Sồi nhọn, Sồi tằm, Sồi mộc châu.

Quercus arbutifolia **Hickel & A.Camus**, Bull. Mus. Natl. Hist. Nat. 29: 598 (1923); Ho, Ill. Fl. Vietnam 2: 656 (2003); Ban, List Pl. Vietnam 2: 261 (2005). —*Cyclobalanopsis obovatifolia* (C.C.Huang) Q.F.Zheng, Acta Phytotax. Sin. 17(3): 118 (1979); Huang et al., Fl. China 4: 387 (1999); Deng et al., Nordic Journal of Botany 29: 208 (2011).

Type: VIETNAM. Nha Trang Province, alt. 1700 m, 20 May 1922, *Poilane 3510* (P [P00379274; P00379275; P00379276], image!).

Specimens examined: VIETNAM. Khanh Hoa Province, alt. 1700 m, 20 May 1922, *Poilane 3510* [ster.] (VNM [VNM00022811!]).

Distribution: China (Guangdong, Hunan) and Vietnam. In Vietnam, this species has been recorded from Quang Tri, Khanh Hoa (Nha Trang, Hòn Heo), Dak Lak (Chư Yang Sin), Lam Dong (Ban 2005).

Phenology: Flowering from May to June.

Ecology: Scattered in the sparse forests, alt. 663–1700 m.

Vernacular name: Sồi vòng.

Quercus asymmetrica **Hickel & A.Camus**, Bull. Mus. Natl. Hist. Nat. 29: 601 (1923); Ho, Ill. Fl. Vietnam 2: 656 (2003); Ban, List Pl. Vietnam 2: 262 (2005). —*Quercus asymmetricus Hick. & Cam.*, —*Quercus patelliformis* Chun, J. Amold Arbor. 28: 241 (1947); —*Cyclobalanopsis*

patelliformis (Chun) Y. C. Hsu & H. W. Jen, J. Beijing Forest. Univ. 15(4): 45 (1993); Huang et al., Fl. China 4: 398 (1999); Deng et al., Novon 20: 400 (2010).

Type: VIETNAM. "Quang Ninh Province", 27 Dec. 1919, *Casabianca 5* (P [P00379266, P00379267], image!).

Specimens examined: VIETNAM. Quang Ninh Province, 27 Dec. 1919, *Casabianca 5* (P [P00379266, P00379267], image!).

Distribution: China (Guangdong, Guangxi, Hainan, S Jiangxi) and Vietnam. In Vietnam, this species has been recorded from Quang Ninh (Ban 2005).

Phenology: Flowering from May to June, fruiting from August to November.

Ecology: Scattered in the sparse forests.

Vernacular name: Sồi quang, Dẻ quang, Sồi lá lệch.

***Quercus chapensis* Hickel & A.Camus**, Bull. Mus. Natl. Hist. Nat. 29: 598 (1923); Ho, Ill. Fl. Vietnam 2: 658 (2003); Ban, List Pl. Vietnam 2: 263 (2005). —*Cyclobalanopsis chapensis* (Hickel & A.Camus) Y.C.Hsu & H.Wei Jen, Acta Phytotax. Sin. 14(2): 78 (1976); Huang et al., Fl. China 4: 394 (1999).

Type: VIETNAM. "Lao Kay, Chapa", *Brillet s.n.* (P [P00753899], image!).

Specimens examined: Lao Cai, Chapa, alt. 1500 m, *Petelot 4836* (VNM [VNM00022857!]).

Distribution: China, Laos. In Vietnam, this species has been recorded from Lao Cai (Fansipan), Cao Bang, Vinh Phuc (Pia Oac), Ha Tay, Khanh Hoa, Lam Dong (Ban 2005).

Phenology: Fruiting from October to December

Ecology: Wet broad-leaved evergreen forests in mountain valleys, alt. 1000-2200m.

Vernacular name: Sồi Sapa, Dẻ Sapa.

***Quercus dankiaensis* A.Camus**, Chênes Atlas 3(2): 51 (1948); Ho, Ill. Fl. Vietnam 2: 659 (2003); Ban, List Pl. Vietnam 2: 264 (2005).

Type: VIETNAM. "Donai Province, Dankia", alt. 1000 m, 29 Jan. 1934, *E. Poilane 23463* (P [P00753903], image!).

Specimens examined: Donai Province, Dankia, alt. 1000 m, 29 Jan. 1934, *E. Poilane 23463* (P [P00753903, image!]).

Distribution: Endemic to Vietnam. In Vietnam, this species has been recorded only from Lam Dong Province (Dan kia ward).
Phenology: Unknown.
Ecology: Scattered in the evergreen forests, alt. 1000–1800 m.
Vernacular name: Sồi Đan kia.

***Quercus edithiae* Skan.**, Hooker's Icon. Pl. 27: t. 2661 (1900); Ho, Ill. Fl. Vietnam 2: 660 (2003); Ban, List Pl. Vietnam 2: 264 (2005). —*Cyclobalanopsis edithiae* (Skan) Schottky, Bot. Jahrb. Syst. 47: 650 (1912); Huang et al., Fl. China 4: 393 (1999).
Type: CHINA. "Hongkong near Tatitin", alt. 152 m, 29 June 1899, *Ford 623* (K [K000832101], image!).
Specimens examined: VIETNAM. Gia Lai Province, Dong Ward, An Khe Pass, 06 May 1978, *s. coll. P2948A* (HN [HN000002991!]).
Distribution: China (Guangdong, Guangxi, Hainan) and Vietnam. In Vietnam, this species has been recorded from North of Vietnam, Gia Lai (Ban 2005).
Phenology: Fruiting from May to December.
Ecology: Broad-leaved evergreen forest on mountain.
Vernacular name: Sồi edith.

***Quercus franchetii* Skan.**, J. Linn. Soc., Bot. 26: 513 (1899); Ban, List Pl. Vietnam 2: 264 (2005); Huang et al., Fl. China 4: 379 (1999); Phengklai, Fl. Thailand 9 (3): 369 (2008).
Type: CHINA. "Yunnan: Mengtze", alt. 1828 m, *Henry 9298* (A [A00034159], image!).
Specimens examined: CHINA. Yunnan Province, alt. 1,828 m, *Henry 9298A* (K [A000832179], image!).
Distribution: China (Yunnam), Thailand (Chiang Mai, Phetchabun, Chaiyaphum). In Vietnam, this species has been recorded from Lao Cai (Sa pa) (Ban 2005).
Phenology: Flowering in April, fruiting from February to September.
Ecology: Upper and lower montane scrubs, mixed deciduous forest, altitude 1100–2600 m.
Vernacular name: Sồi Vân nam.

***Quercus glauca* Thunb.**, Syst. Veg. ed. 14 (J. A. Murray): 858 (1784); Ho, Ill. Fl. Vietnam 2: 661 (2003); Ban, List Pl. Vietnam 2: 265 (2005). —*Cyclobalanopsis glauca* (Thunb.) Oerst, Vidensk. Meddel. Dansk Naturhist. Foren. Kjøbenhavn 1866: 78 (1866); Huang et al., Fl. China 4: 396 (1999).

Type: JAPAN (n.v).

Specimens examined: Vietnam, Son La Province, *s. coll. s. n.* (HN [HN0000030338!]).

Distribution: China (Anhui, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Sichuan, Taiwan, Xizang, Yunnan, Zhejiang), Japan and Vietnam. In Vietnam, this species has been recorded from Son La, Gia Lai, Ninh Thuan (Ban 2005).

Phenology: Flowering from April to May, fruiting in October.

Ecology: Broad-leaved evergreen forests and mixed mesophytic forest on mountain slopes or in valleys.

Vernacular name: Sồi sim.

***Quercus griffithii* Hook. f. & Thomson ex Miq.**, Ann. Mus. Bot. Lugduno-Batavi 1: 104 (1863); Ban, List Pl. Vietnam 2: 266 (2005); Huang et al. Fl. China 4: 373 (1999). —*Quercus aliena subsp. griffithii* (Hook.f. & Thomson ex Miq.), Phengklai, Phengklai, Fl. Thailand 9(3): 359 (2008).

Type: INDIA. "Khasia, region temperate", *Hooker & Thomson s. n.* (GH [GH01153285], image!).

Specimens examined: LAOS. Paksong Province, alt. 1,200 m, *Poilane 15690* (VNM [VNM00020136!]).

Distribution: India, China (Guizhou, Sichuan, Xizang, Yunnan), Myanmar, Thailand (Phetchabun, Loei, Chaiyaphum), Laos and Vietnam. In Vietnam, this species has been recorded from Lai Chau, Son La, Lao Cai (Ban 2005).

Phenology: Fruiting in May

Ecology: Dry upper mixed pine-spread leaf forests, alt. 800–1200 m.

Vernacular name: Sồi lá to, Sồi Griffith.

***Quercus lineata* Blume**, Bijdr. Fl. Ned. Ind. 10: 523 (1826); Phengklai, Fl. Thailand 9 (3): 381 (2008); Li et al., Phytotaxa 226(3): 226 (2016).

Type: INDONESIA. (n.v.).

Distribution: China (Hainan), Thailand (Mae Hong Son, Chiang Mai, Chiang Rai, Loei, Nakhon Si Thammarat), Vietnam (Ninh Binh).
Phenology: Flowering from April to May, fruiting from September to November.
Ecology: In lower to middle upper montane forest, at alt. 1000–2000 m, on yellow sandy soil or limestone areas (Li et al. 2016).
Vernacular name: Sồi Li nê.

***Quercus mespilifolia* Wall.**, Prodr. [A. P. de Candolle]. 16(2): 101 (1864); Ho, Ill. Fl. Vietnam 2: 663 (2003); Phengklai, Fl. Thailand 9(3): 383–385 (2008). —*Quercus mespilifolia var. pubescens* Barnett ex Smitinand & Phengklai, Thai Forest Bull., Bot. 32: 119 (2004); —*Quercus mespilifolia var. mespilifolia* Phengklai, Fl. Thailand 9 (3): 383–385 (2008).
Type: MYANMAR. "Prome montane", *Wallich 2766* (K [K001117049], image!).
Distribution: Myanma, Thailand (Mae Hong Son, Chiang Mai, Chiang Rai, Nan, Phrae, Uttaradit, Tak, Phetchabun, Loei, Nakhon Ratchasima, Kanchanaburi, Uthai Thani, Chanthaburi) and Vietnam. In Vietnam, this species has been recorded from Son La, Lam Dong (Ban 2005).
Phenology: Flowering from February to September, fruiting from January to September.
Ecology: Pine-oak forest and dry evergreen forest, alt. 700–1000 m.
Vernacular name: Sồi lá nhót.

***Quercus petelotii* A.Camus**, Bull. Mus. Natl. Hist. Nat. sér. 2, 23: 435 (1951); Ho, Ill. Fl. Vietnam 2: 663 (2003); Ban, List Pl. Vietnam 2: 268 (2005).
Type: VIETNAM. "Tonkin: Sentier forestier entre la garderie de Lo Qui Ho et Ta Phinh", *Pételot 7481* (P [P00753993], image!).
Specimens examined: Lao Cai Province, Lo Qui Ho, Aug. 1935, *Pételot 7482* (P [P00753992], image!).
Distribution: Endemic to Vietnam. In Vietnam, this species has been recorded from Lai Chau (Ta Phinh), Lao Cai (Sapa, O Quy Ho) (Ban 2005).
Phenology: Flowering in August, fruiting in March.
Ecology: Scattered in evergreen forests, alt. 200–1700 m.
Vernacular name: Sồi petelot.

***Quercus rex* Helsm.**, Hooker's Icon. Pl. 27: t. 2663 (1899); Ho, Ill. Fl. Vietnam 2: 664 (2003); Ban, List Pl. Vietnam 2: 266 (2005); Phengklai, Fl. Thailand 9(3): 397 (2008). —*Cyclobalanopsis rex* (Hemsl.) Schottky, Bot. Jahrb. Syst. 47: 651 (1912); Huang et al., Fl. China 4: 390 (1999). *Type*: CHINA. "Szemao, Yunnan", alt. 1219 m, *Henry 12665* (MO [MO-204638], A [A00034184], image!).

Distribution: China (Yunnan), Thailand (Chiang Mai, Tak), Laos and Vietnam. In Vietnam, this species has been recorded from Lam Dong (Ban 2005).

Phenology: Flowering from March to December, fruiting in July.

Ecology: Lower montane forest and evergreen forest, alt. 800–1600 m.

Vernacular name: Sồi thầy

***Quercus rupestris* Hickel & A.Camus**, Bull. Mus. Natl. Hist. Nat. 1923, 29: 599 (1923); Ho, Ill. Fl. Vietnam 2: 664 (2003); Ban, List Pl. Vietnam 2: 269 (2005).

Type: VIETNAM. "Annam: Núi han heo près de Nhatrang", alt. 700 m, 29 Dec. 1922, *Poilane 4817* [fr.] (P [P00379243], image!; VNM [VNM00019960!).

Distribution: Endemic to Vietnam (Khanh Hoa).

Phenology: Flowering and fruiting from April to December.

Ecology: Scattered in lower forests, alt. 400–900 m.

Vernacular name: Sồi vùng đá.

***Quercus thorelii* Hickel & A.Camus**, Bull. Mus. Natl. Hist. Nat. 1923, 29: 599 (1923); Ho, Ill. Fl. Vietnam 2: 665 (2003); Ban, List Pl. Vietnam 2: 270 (2005). —*Cyclobalanopsis thorelii* (Hickel & A.Camus) Hu, Bull. Fan Mem. Inst. Biol. 10: 106 (1940); Huang et al., Fl. China 4: 394 (1999). *Type:* LAOS. "Múông Pun, province de Fan Neúa", alt. 600–700 m, 17 Sep. 1920, *Poilane 1902* (P [P00379271], image!).

Specimens examined: VIETNAM. Vinh Province, Khe Nhe, 5 Aug. 1929, *Poilane 16582* (VNM [VNM00019974!]).

Distribution: Laos, China (Guangxi, Yunnan) and Vietnam. In Vietnam, this species has been recorded from Gia Lai, Nghe An (Ban 2005).

Phenology: Flowering in April, fruiting from September to October

Ecology: Scattered in sparse forest, alt. 200–1000 m.

Vernacular name: Sồi Thorel.

***Quercus variabilis* Blume**, Mus. Bot. 1(19): 297 (1851); Huang et al., Fl. China 4: 372 (1999); Ban, List Pl. Vietnam 2: 270 (2005).

Type: JAPAN. (n.v.).

Specimens examined: JAPAN. Tsu-sima Island, May 1895, *Wilford 570* (K [000832111]).

Distribution: This species occurs in China (Anhui, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Liaoning, Shaanxi, Shandong, Shanxi, Sichuan, Taiwan, Yunnan, Zhejiang), Japan, North Korea and Vietnam. In Vietnam, this species has been recorded from Lao Cai, Son La (Moc Chau), Ha Giang (Dong Van), Hoa Binh (Mai Chau), Tuyen Quang (Ban 2005).

Phenology: Fruiting in October

Ecology: Mixed broad-leaved forests and pine-forest or valleys on loamy soil, loamy sandy soil.

Vernacular name: Sồi đen, Dẻ bần.

Doubtful species

***Quercus gemelliflora* Blume.**, Verh. Batav. Genootsch. Kunsten 9: 222 (1825); Ban, List Pl. Vietnam 2: 265 (2005). —*Quercus gemmiflora* Blume, Ho, Ill. Fl. Vietnam 2: 660 (2003).

Type: INDONESIA. (n.v.).

Note: This species was described from Indonesia. In Vietnam, Ho (2003) and Ban (2005) reported this species from Ha Coi, Quang Ninh, Hoa Binh, Ha Tay. However, *Q. gemelliflora* is not described in the Flora of China (Huang et al. 1999) and the Flora of Thailand (Phengklai 2008). Disjunction between Indonesia and northern Vietnam seems unlikely and we need further careful studies to confirm whether this species is really distributed in Vietnam.

***Quercus gomeziana* A.Camus**, Bull. Mus. Natl. Hist. Nat. 1931, 2, 3: 338 (1931); Ho, Ill. Fl. Vietnam 2: 661 (2003); Ban, List Pl. Vietnam 2: 265 (2005).

Type: not seen.

Note: This species was described by Camus (1931), but its type specimens were not found in any herbarium. Also, its original description was not found. In Vietnam, this species was reported from Lam Dong Province by Ho (2003) and Ban (2005), but I did not find any specimens in herbarium

of the Institute of Ecology and Biological Resources (HN herbarium), VNM herbarium (VNM herbarium), and DLU herbarium (DLU herbarium placed in Dalat University, Lam Dong Provinces). Also, I have not seen the original description yet. Besides, *Q. gomeziana* is not reported in the the Flora of China (Huang et al. 1999) and the Flora of Thailand (Phengklai 2008). Thus, we need further careful studies to confirm whether this species is distributed in Vietnam.

***Quercus oblongata* D. Don**, Prodr. Fl. Nepal: 57 (1825). —*Quercus leucotrichophora* A.Camus, Rivièra Sci. 22: 66 (1935), nom. nud. [basionym: Quercus incana Roxb.]; Ho, Ill. Fl. Vietnam 2: 663 (2003). —*Quercus leucotrichophora* A.Camus ex Bahadur Indian Forester 101: 101 (1975), nom. illeg. [basionym: *Quercus incana* Roxb.]. —*Quercus incana* Roxb., Fl. Ind. ed. 3: 642 (1832), nom. illeg.; Ban, List Pl. Vietnam 2: 266 (2005).

Type: INDIA. (n.v.).

Note: Quercus incana Roxb. is a later homonym of *Quercus incana* Bartram described in 1791 for an American species. Bahadur (1975) tried to validate the name "*Quercus leucotrichophora* A.Camus" that was not effectively published, but his name "*Quercus leucotrichophora* A.Camus ex Bahadur" is illegitimate because it is based on an illegitimate name *Quercus incana* Roxb. Thus, *Quercus oblongata* D. Don in Prodr. Fl. Nepal.: 57 (1825) is the only valid name for this species common in India, although it is described based on sterile material and the type specimen is unknown (Bahadur, 1975). Ho (2003) and Ban (2005) reported this species from Nhatrang, but this species is not documented in the Flora of China (Huang et al., 1999) and the Flora of Thailand (Phengklai, 2008). Disjunction between India and Nhatrang seems unlikely and we need further careful studies to confirm whether this species is distributed in Vietnam.

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Legends

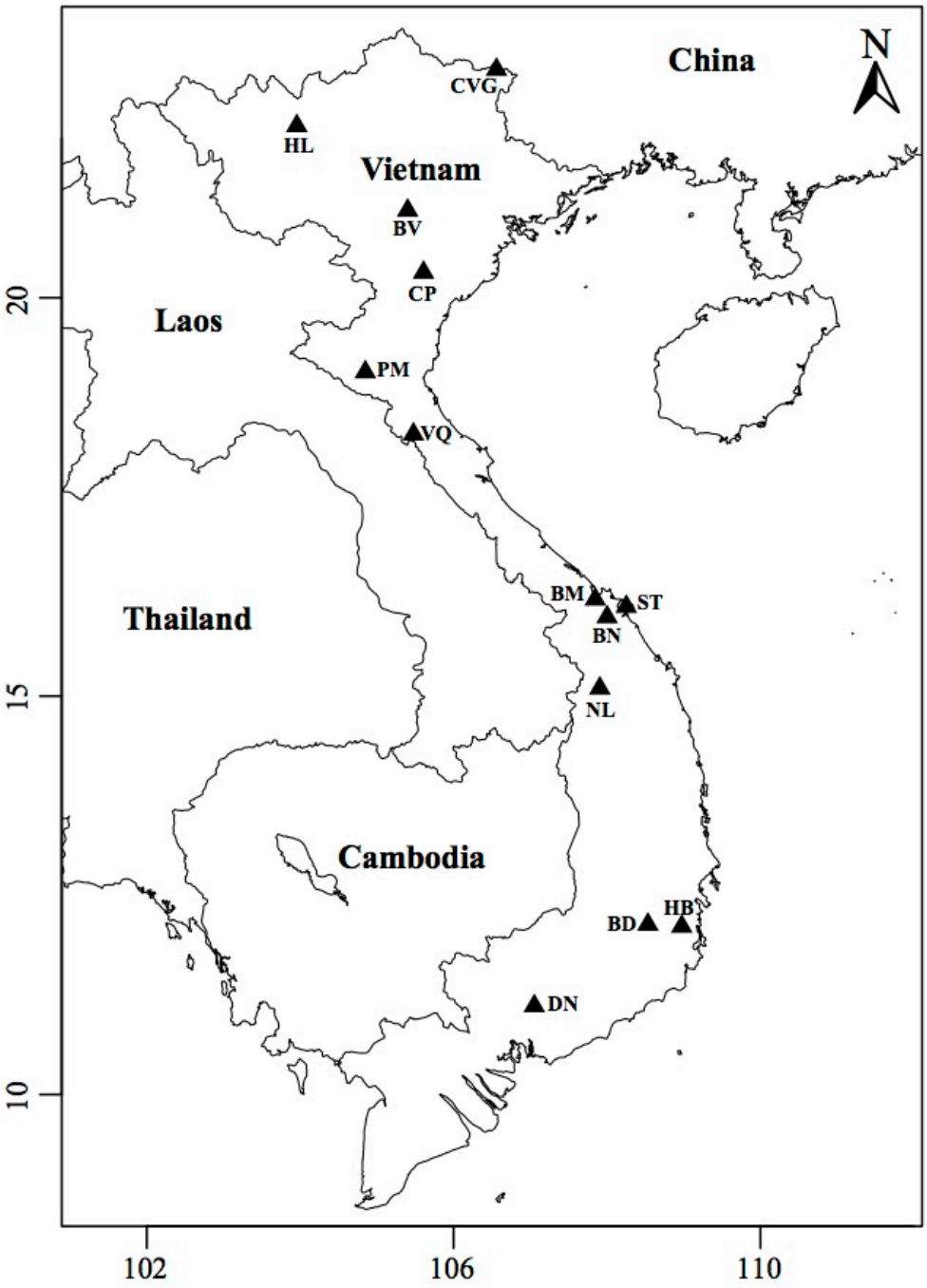

Figure 4.1. Collection sites in Vietnam in this study: CVG (Cao Vit Gibbon CA), HL (Hoang Lien NP), BV (Ba Vi NP), CP (Cuc Phuong NP), PM (Pu Mat NP), VQ (Vu Quang NP), BM (Bach Ma NP), ST (Son Tra CA), BN (Ba Na NR), NL (Ngoc Linh NR), BD (Bidoup-Nui Ba NP), HB (Hon Ba NR), DN (Dong Nai NR).

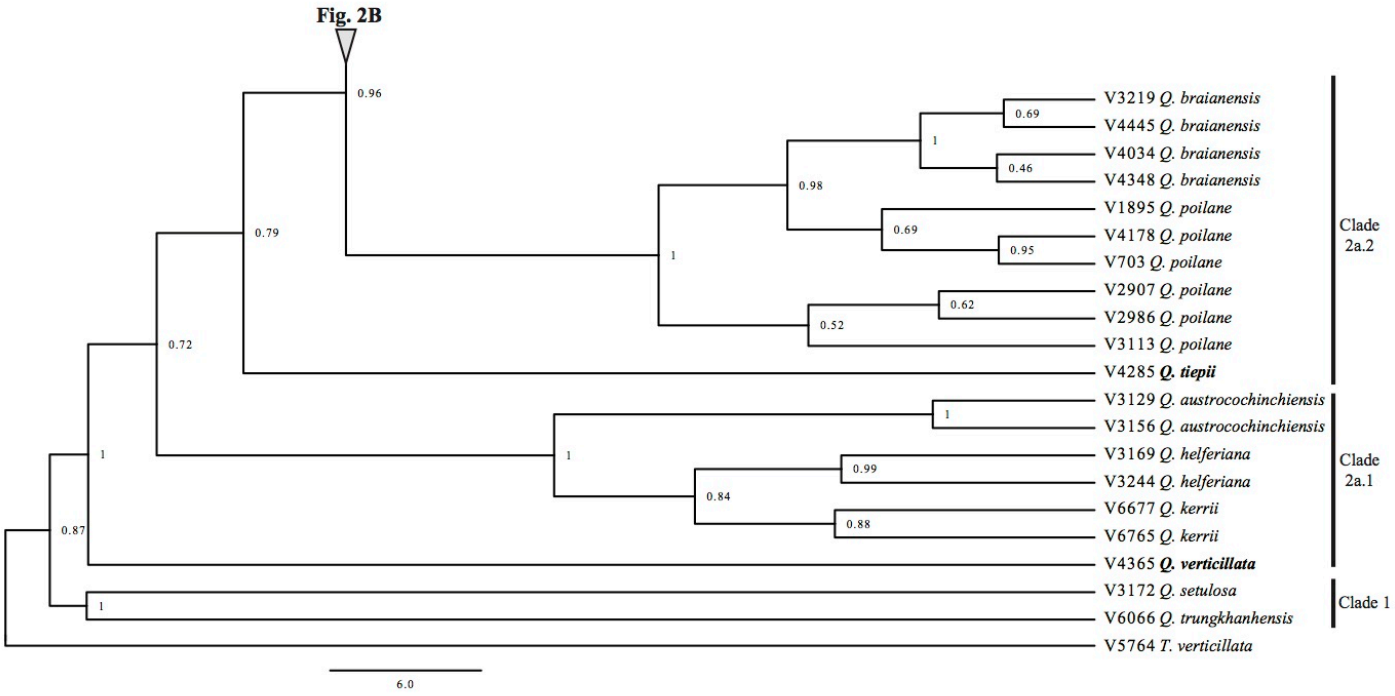

Figure 4.2A. Bayesian phylogeny of 20 samples of *Quercus* and one *Trigonobalanus* (outgroup) based on *rbc*L, *mat*K and ITS sequences. Braches are labeled with posterior probability.

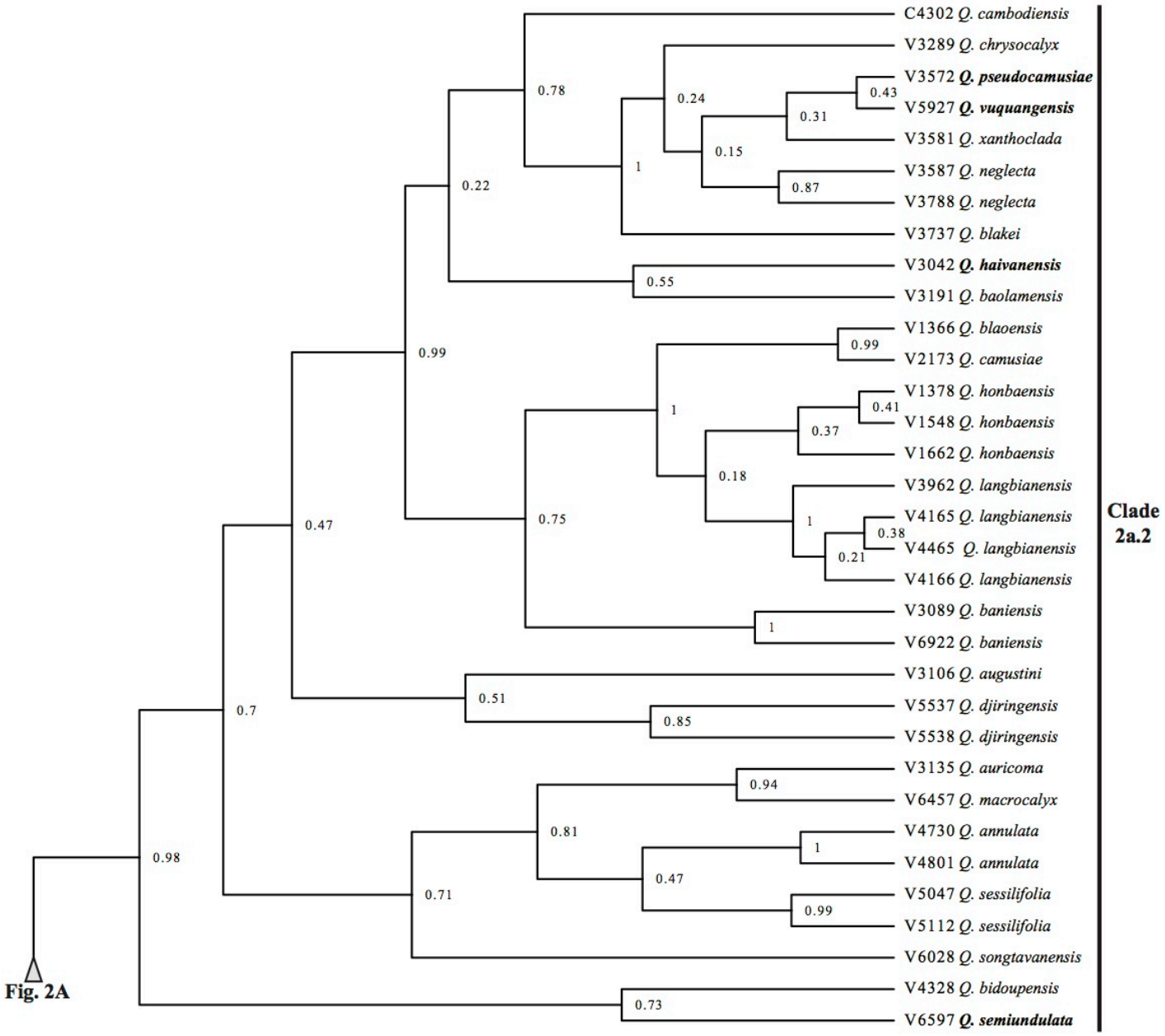

Figure 4.2B. Bayesian phylogeny of 33 samples of *Quercus* based on *rbc*L, *mat*K and ITS sequences. Braches are labeled with posterior probability.

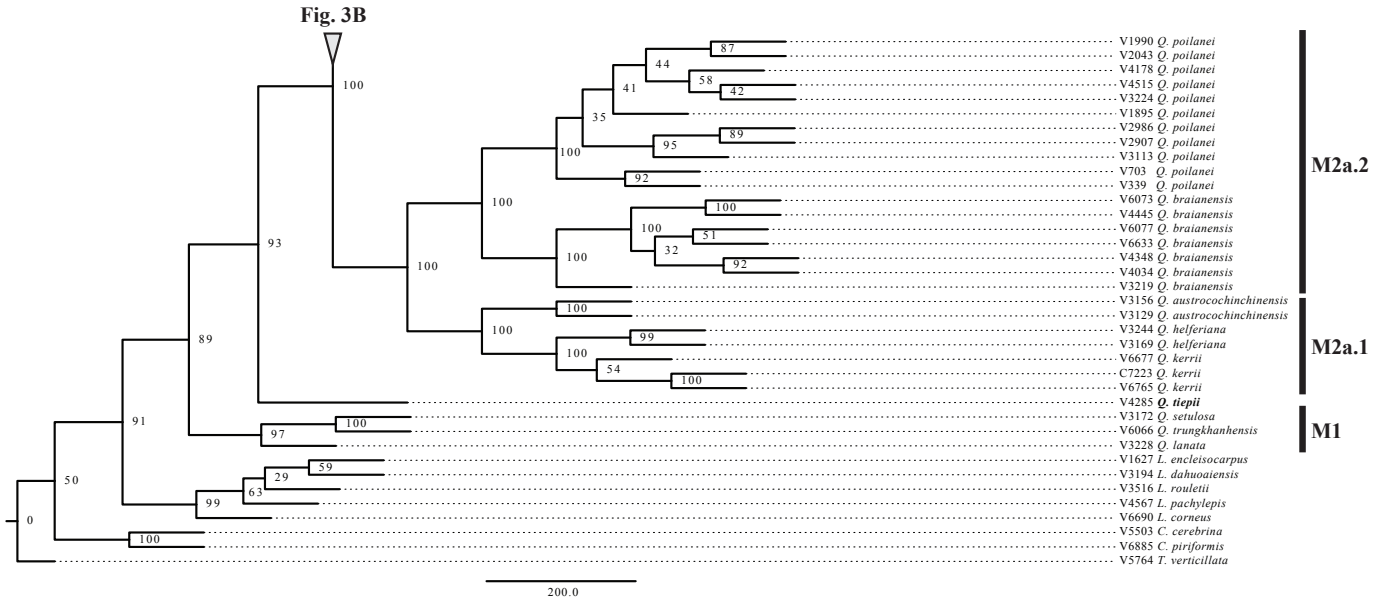

Figure 4.3A. MIG-seq tree (NJ tree) of 29 *Quercus* samples and outgroups (based on presence/absence data of 35,259 MIG-seq loci for 95 *Quercus* samples). Branches are labeled with bootstrap support (% of 1000 replicates).

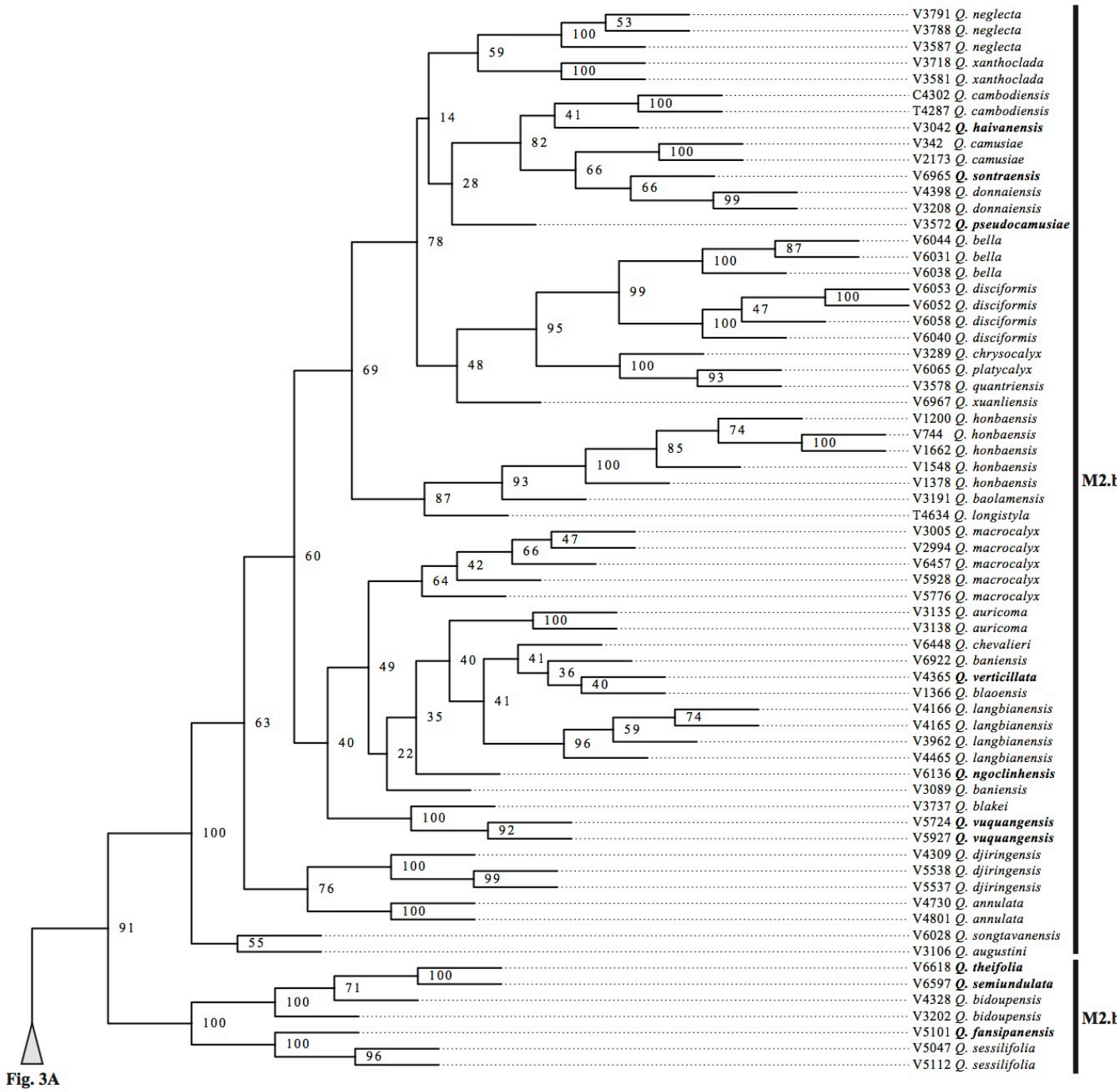

Figure 4.3B. MIG-seq tree (NJ tree) of 66 *Quercus* samples (based on presence/absence data of 35,259 MIG-seq loci for 95 *Quercus* samples). Branches are labeled with bootstrap support (% of 1000 replicates).

Table 4.1. Plant materials of *Quercus* and outgroups collected in Vietnam and used in this study (including samples from Cambodia, Thailand).

Taxon	Locality of voucher	Voucher No.	GenBank accession number			MIG-seq
			*rbc*L	*mat*K	ITS	
Q. annulata	Hoang Lien NP, Lao Cai Prov.	V4730	LC318796*	LC318516*	MF770291*	+
Q. annulata	Hoang Lien NP, Lao Cai Prov.	V4801	###	###	MG549021	+
Q. augustinii	Ba Na NR, Da Nang Prov.	V3106	###	###	MG549004	+
Q. auricoma	Son Tra CA, Da Nang Prov.	V3135	LC318778*	LC318498*	MF770277*	+
Q. auricoma	Son Tra CA, Da Nang Prov.	V3138	LC318779*	LC318499*	-	+
Q. austrocochinchinensis	Son Tra CA, Da Nang Prov.	V3129	LC318777*	LC318497*	MF770276*	+
Q. austrocochinchinensis	Son Tra CA, Da Nang Prov.	V3156	LC318780*	LC318500*	MF770278*	+
Q. baniensis	Ba Na NR, Da Nang Prov.	V3089	LC318775*	LC318495*	MF770274*	+
Q. baniensis	Ba Na NR, Da Nang Prov.	V6922	LC318802*	LC318522*	MF770296*	+
Q. baolamensis	Bao Lam, Lam Dong Prov.	V3191	LC318782*	LC318502*	MF770280*	+
Q. bella	Ba Vi NP, Ha Noi Capital	V6031	-	-	-	+
Q. bella	Ba Vi NP, Ha Noi Capital	V6038	LC331259	LC331256	-	+
Q. bella	Ba Vi NP, Ha Noi Capital	V6044	-	-	-	+
Q. bidoupensis	Lan Tranh, Lam Dong Prov.	V3202	LC318783*	LC318503*	-	+
Q. bidoupensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4328	LC318793*	LC318513*	MF770288*	+
Q. blakei	Vu Quang NP, Ha Tinh Prov.	V3737	###	###	MG549012	+
Q. blaoensis	Hon Ba NR, Khanh Hoa Prov.	V1366	LC318768*	LC318488*	MF770269*	+
Q. braianensis	Lan Tranh, Lam Dong Prov.	V3219	LC318785*	LC318505*	MF770281*	+
Q. braianensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4034	###	###	MG549014	+
Q. braianensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4348	###	###	MG549018	+
Q. braianensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4445	LC318794*	LC318514*	MF770289*	+
Q. braianensis	Ngoc Linh NR, Kon Tum Prov.	V6073	-	-	-	+
Q. braianensis	Ngoc Linh NR, Kon Tum Prov.	V6077	-	-	-	+
Q. braianensis	Ngoc Linh NR, Kon Tum Prov.	V6633	-	-	-	+
Q. cambodiensis	Bokor NP, Kampot, Cabodia	C4302	LC318766*	LC318445*	MF770268*	+
Q. cambodiensis	Phu Kradueng NP, Thailand	T4287	-	-	-	+
Q. camusiae	Hon Ba NR, Khanh Hoa Prov.	V342	LC318787*	LC318507*	-	+
Q. camusiae	Hon Ba NR, Khanh Hoa Prov.	V2173	LC318773*	LC318493*	MF770272*	+
Q. chevalieri	Ngoc Linh NR, Kon Tum Prov.	V6448	-	-	-	+
Q. chrysocalyx	Vu Quang NP, Ha Tinh Prov.	V3289	###	###	MG549007	+
Q. donnaiensis	Cong Troi, Lam Dong Prov.	V3208	LC318784*	LC318504*	-	+
Q. donnaiensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4398	-	-	-	+

Q. disciformis	Ba Vi NP, Ha Noi Capital	V6040	-	-	-	+
Q. disciformis	Ba Vi NP, Ha Noi Capital	V6052	-	-	-	+
Q. disciformis	Ba Vi NP, Ha Noi Capital	V6053	-	-	-	+
Q. disciformis	Ba Vi NP, Ha Noi Capital	V6058	LC331258	LC331255	-	+
Q. djiringensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4309	-	-	-	+
Q. djiringensis	Lam Dong, Prov.	V5537	LC318797*	LC318517*	MF770292*	+
Q. djiringensis	Lam Dong, Prov.	V5538	LC318798*	LC318518*	MF770293*	+
Q. helferiana	Da Lat, Lam Dong Prov.	V3169	LC318781*	LC318501*	MF770279*	+
Q. helferiana	Bidoup-Nui Ba NP, Lam Dong Prov.	V3244	LC318786*	LC318506*	MF770282*	+
Q. honbaensis	Hon Ba NR, Khanh Hoa Prov.	V744	-	-	-	+
Q. honbaensis	Hon Ba NR, Khanh Hoa Prov.	V1200	LC318767*	LC318446*	-	+
Q. honbaensis	Hon Ba NR, Khanh Hoa Prov.	V1378	LC318769*	LC318489*	MF770270*	+
Q. honbaensis	Hon Ba NR, Khanh Hoa Prov.	V1548	LC318770*	LC318490*	MG548998	+
Q. honbaensis	Hon Ba NR, Khanh Hoa Prov.	V1662	LC318771*	LC318491*	MG548999	+
Q. kerrii	Ngoc Linh NR, Kon Tum Prov.	V6765	LC318801*	LC318521*	MF770295*	+
Q. kerrii	Ngoc Linh NR, Kon Tum Prov.	V6677	###	###	MG549033	+
Q. kerrii	Sen Monorom, Cambodia	C7223	-	-	-	+
Q. lanata	Bidoup-Nui Ba NP, Lam Dong Prov.	V3228	-	-	-	+
Q. langbianensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V3962	LC318790*	LC318510*	MF770285*	+
Q. langbianensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4165	LC318791*	LC318511*	MF770286*	+
Q. langbianensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4166	LC318792*	LC318512*	MF770287*	+
Q. langbianensis	Bidoup-Nui Ba NP, Lam Dong Prov.	V4465	LC318795*	LC318515*	MF770290*	+
Q. longistyla	Phu Kradueng NP, Thailand	T4634	-	-	-	+
Q. macrocalyx	Bach Ma NP, Thua Thien Hue Prov.	V2994	-	-	-	+
Q. macrocalyx	Bach Ma NP, Thua Thien Hue Prov.	V3005	-	-	-	+
Q. macrocalyx	Vu Quang NP, Ha Tinh Prov.	V5776	-	-	-	+
Q. macrocalyx	Vu Quang NP, Ha Tinh Prov.	V5928	-	-	-	+
Q. macrocalyx	Ngoc Linh NR, Kon Tum Prov.	V6457	LC318800*	LC318520*	MF770294*	+
Q. neglecta	Vu Quang NP, Ha Tinh Prov.	V3587	LC318788*	LC318508*	MF770283*	+
Q. neglecta	Vu Quang NP, Ha Tinh Prov.	V3788	LC318789*	LC318509*	MF770284*	+
Q. neglecta	Vu Quang NP, Ha Tinh Prov.	V3791	-	-	-	+
Q. platycalyx	Vu Quang NP, Ha Tinh Prov.	V6065	-	-	-	+
Q. poilanei	Hon Ba NR, Khanh Hoa Prov.	V339	-	-	-	+
Q. poilanei	Hon Ba NR, Khanh Hoa Prov.	V703	###	###	MG549034	+
Q. poilanei	Bidoup-Nui Ba NP, Lam Dong Prov.	V1895	LC318772*	LC318492*	MF770271*	+
Q. poilanei	Hon Ba NR, Khanh Hoa Prov.	V1990	-	-	-	+

Q. poilanei	Hon Ba NR, Khanh Hoa Prov.	V2043	-	-	-	+
Q. poilanei	Bach Ma NP, Thua Thien Hue Prov.	V2907	###	###	MG549002	+
Q. poilanei	Bach Ma NP, Thua Thien Hue Prov.	V2986	LC318774*	LC318494*	MF770273*	+
Q. poilanei	Ba Na NR, Dang Nang Prov.	V3113	LC318776*	LC318496*	MF770275*	+
Q. poilanei	Bidoup-Nui Ba NP, Lam Dong Prov.	V3224	-	-	-	+
Q. poilanei	Bidoup-Nui Ba NP, Lam Dong Prov.	V4178	###	###	MG549015	+
Q. poilanei	Bidoup-Nui Ba NP, Lam Dong Prov.	V4515	-	-	-	+
Q. quangtriensis	Vu Quang NP, Ha Tinh Prov.	V3578	-	-	-	+
Q. sessilifolia	Hoang Lien NP, Lao Cai Prov.	V5047	###	###	MG549022	+
Q. sessilifolia	Hoang Lien NP, Lao Cai Prov.	V5112	###	###	MG549023	+
Q. setulosa	Duc Trong, Lam Dong Prov.	V3172	###	###	MG549005	+
Q. songtavanensis	Cuc Phuong NP, Ninh Binh Prov.	V6028	###	###	MG549026	+
Q. trungkhanhensis	Cao Vit Gibbon CA, Cao Bang Prov.	V6066	###	LC258443	KY867547	+
Q. xuanlienensis	Xuan Lien NR, Thanh Hoa Prov.	V6967	LC331257	LC331254	-	+
Q. xanthoclada	Vu Quang NP, Ha Tinh Prov.	V3718	###	###	-	+
Q. xanthoclada	Vu Quang NP, Ha Tinh Prov.	V3581	###	###	MG549011	+
Q. semiundulata	Ngoc Linh NR, Kon Tum Prov.	V6597	###	###	MG549030	+
Q. theifolia	Ngoc Linh NR, Kon Tum Prov.	V6618	-	-	-	+
Q. fansipanensis	Hoang Lien NR, Lao Cai Prov.	V5101	-	-	-	+
Q. haivanensis	Hai Van Pass, Da Nang Prov.	V3042	###	###	MG549003	+
Q. pseudocamusiae	Vu Quang NP, Ha Tinh Prov.	V3572	###	###	MG549010	+
Q. vuquangensis	Vu Quang NP, Ha Tinh Prov.	V5724	-	-	-	+
Q. vuquangensis	Vu Quang NP, Ha Tinh Prov.	V5927	###	###	MG549025	+
Q. verticillata	Bidoup-Nui Ba NP, Lam Dong Prov.	V4365	###	###	MG549019	+
Q. ngoclinhensis	Ngoc Linh NR, Kon Tum Prov.	V 6136	LC318799*	LC318519*	-	+
Q. tiepii	Bidoup-Nui Ba NP, Lam Dong Prov.	V4285	###	###	MG549016	+
Q. sontraensis	Son Tra CA, Da Nang Prov.	V6965	-	-	-	+
Castanopsis cerebrina	Pu Mat NP, Nghe An Prov.	V5503	x	x	x	+
Castanopsis piriformis	Hon Ba NR, Khanh Hoa Prov.	V1627	x	x	x	+
Lithocarpus corneus	Ngoc Linh NR, Kon Tum Prov.	V6690	x	x	x	+
Lithocarpus dahuoaiensis	Chuoi Pass, Dahuoai, Lam Dong Prov.	V3194	x	x	x	+
Lithocarpus encleisocarpus	Hon Ba NR, Khanh Hoa Prov.	V1627	x	x	x	+
Lithocarpus pachylepis	Hoang Lien NP, Lao Cai Prov.	V4567	x	x	x	+
Lithocarpus rouletii	Vu Quang NP, Ha Tinh Prov.	V3516	x	x	x	+
Trigonobalanus verticillata	Vu Quang NP, Ha Tinh Prov.	V5764	LC318965*	LC318549*	MF770380*	+

(+): Using to analyze in this study; (-): Do not successful sequencing; (*): From GenBank; (x): Do not using in this study; (###): Submitting to GenBank

Table 4.2. List of primers used for amplification and sequencing of two DNA regions.

DNA region	Primer	Sequence (5' to 3')	Reference
*mat*K	*mat*K-XF	TAATTTACGATCAATTCATTC	Ford et al. 2009
	*mat*K-1326R	TCTAGCACACGAAAGTCGAAGT	Cuénoud et al. 2002
*rbc*L	*rbc*La-F	ATGTCACCACAAACAGAGACTAAAGC	Levin 2003
	*rbc*L-724r	TCGCATGTACCTGCAGTAGC	Fay et al. 1997
ITS	ITS-18F	GTCCACTGAACCTTATCATTTAGAGG	Rohwer et al. 2009
	ITS-26R	GCCGTTACTAAGGGAATCCTTGTTAG	Rohwer et al. 2009

Table 4.3. Summary statistics of datasets used for phylogenetic inference comprising *rbc*L*, mat*K and ITS sequences of 54 samples of *Quercus* and *Trigonobalanus verticillata* (outgroup).

Regions	*rbc*L	*mat*K	ITS	Combined data
Aligned sequence length	657	834	543	2,034
Variable DNA sites	12	47	142	204
Parsimony-informative sites	3	15	57	78