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

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Ph.D. Thesis

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

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Contents

Preface

This dissertation is the main outcome of my doctoral program under the supervision of Professor Tetsukazu Yahara in Laboratory of Ecological Science, Graduate School of Systems Life Sciences, Kyushu University. This research was granted by Project no. 911 of Vietnam International Education and Development (VIED), Ministry of Education and Training of Vietnam and also supported by the Environment Research and Technology Development Fund (S9, 4-1601) of the Ministry of the Environment, Japan.

My study focuses on the taxonomic study of *Lithocarpus* (Fagaceae) in Vietnam based on molecular phylogeny and morphological observations using our new collections from Vietnam and its surrounding countries. Through five chapters of this thesis, I reviewed three complexes of *Lithocarpus*, *L. elegans* complex, *L. hancei* complex, and *L. vestitus* complex and recognized nine new species. The photographs, illustrations, DNA barcodes of the sequences of *rbcL*, *matK* and ITS, and preliminary conservation assessments are provided for these new species. In addition, eight species are lectotypified in this study.

In Chapter I, I report *Lithocarpus dahuoaiensis* Ngoc & L.V.Dung which we discovered from our field surveys in Lam Dong Province, Central Highland of Vietnam. This new species was published in the PhytoKeys (doi: 10.3897/phytokeys.69.9821).

In Chapter II, I report two new species, *Lithocarpus hongiaoensis* Ngoc & Binh and *Lithocarpus bidoupensis* Ngoc & Tagane, which were found during our floristic inventories in Bidoup-Nui Ba National Park, Lam Dong Province in 2015–2016.

In Chapter III, I report *Lithocarpus vuquangensis* Ngoc & Hung from Vu Quang National Park, North Central of Vietnam. It was recognized by the morphological comparison and phylogenyetic study based on *rbcL*, *matK* and ITS.

In Chapter IV, I examine the taxonomy of the *Lithocarpus vestitus* complex and its close relatives in Vietnam, Laos, Cambodia, and Thailand using our field observations,

morphological studies of dried specimens in various herbaria, and molecular analyses. In our molecular approach, we employed genome-wide sequences obtained using next-generation sequencing (MIG-seq; Suyama and Matsuki 2015) to reconstruct phylogenetic relationships among species in the *L. vestitus* complex. Based on the evidence from morphological observations and the MIG-seq-based phylogenetic tree, we recognized 13 species in the *L. vestitus* complex in the four countries including three new species: *L. chinhii* Ngoc & Binh, *L. pierreioides* Ngoc, Tagane & Yahara, and *L. pseudoannamensis* Ngoc & Binh.

In Chapter V, I describe results from molecular phylogenetic analyses and morphological observations and revise the taxonomy of the *Lithocarpus elegans* complex, the *L. hancei* complex and their closely related species in Southeast Asia. The results supported that six and four species are distinguished in the *L. elegans* complex and the *L. hancei* complex, respectively. In the *L. elegans* complex, two new species, *L. bokorensis* Ngoc, Tagane & Yahara, and *L. monoromensis* Ngoc, Tagane & Yahara. were recognized from Cambodia.

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Abstract

Lithocarpus is the second largest genus of the family Fagaceae, with approximately 350 species in the world. The centre of species diversity is mainly in continental Southeast Asia, especially in China (123 spp.) and Vietnam (117 spp.). In Vietnam, almost all the species of *Lithocarpus* were described by French botanists until 1920s and some other were until 1950s. Since then, the taxonomic inventories of *Lithocarpus* in Vietnam were disrupted until recently. Therefore, updating our taxonomic knowledge on the species of *Lithocarpus* using new collections and new techniques such as phylogenetic analysis based on DNA markers are required.

In this dissertation, our morphological observations and molecular phylogenetic analyses based on both classic DNA sequencing of two cpDNA (*rbc*L and *mat*K) and one nrDNA (ITS) regions and multiplexed inter-simple sequence repeat genotyping by sequencing (MIG-seq) were employed to revise three complexes of *Lithocarpus* in Vietnam: *L. elegans* complex, *L. hancei* complex, and *L. vestitus* complex.

The NJ tree based on MIG-seq data provided the evidence of species delamination. Among species of the *L. elegans* complex, *L. blaoensis* was clustered not with the other species of the *L. elegans* complex and more close to the *L. hancei* complex. Three other species previously reduced to or identified as *L. elegans* were distinct and an additional new species was confirmed. Thus, "*L. elegans s. lat.*" previously considered as a widespread polymorphic species included at least five distinct species.

In the *L. hancei* complex, both the MIG-seq tree and a population genetic analysis supported that it includes at least three distinct species. However, there was no diagnostic morphological difference between *L. hancei* and *L. jacksonianus* in spite that the two species co-occur in the same habitat of northern Vietnam. The third species *L. yersinii* distributed in souther Vietnam was morphologically distinct.

In the *L. vestitus* complex, the MIG-seq tree and morphological observations showed that "*L. vestitus s. lat.*" previously considered as a widespread polymorphic species included many cryptic species. As a result, we recognized 13 distinct species including three new species.

Through our study, nine new species are recognized and described: *L. bidoupensis,* sp. nov., *L. chinhii,* sp. nov., *L. dahuoaiensis* sp. nov., *L. hongiaoensis,* sp. nov., *L. pseudoannamensis,* sp. nov., and *L. vuquangensis,* sp. nov. from Vietnam, and *L. bokorensis,* sp. nov. *L. monoromensis,* sp. nov., and *L. pierreioides,* sp. nov. from Cambodia. The photographs, illustrations, DNA barcode sequences, and the description of preliminary conservation status are also provided for the new species. Additionally, eight species are lectotypified in this study.

By applying both molecular and morphological approaches, we could resolve a long-standing problem on the taxonomy of polymorphic species complexes of *Lithocarpus* in Vietnam.

Chapter I

Lithocarpus dahuoaiensis (Fagaceae), a new species from Lam Dong Province, Vietnam

Abstract

Lithocarpus dahuoaiensis Ngoc & L.V. Dung, a new species from the Central highland of Vietnam, is described and illustrated. The new species is morphologically similar to *Lithocarpus macphailii* (M. R. Hend.) Barnett or *Lithocarpus encleisocarpus* (Korth.) A. Camus in having completely entire leaf margin, solitary cupule, long stalks of fruits, deeply cup-shaped or turbinate cupules, with a number of horizontal filiform lines. The species differs in its nut enclosure ca. 1/2 – 2/3 of the nut, adaxially glabrous leaf blades, secondary veins 11–12 pairs and faintly to very faintly visible hairs on the outside of the cupule. A table showing the morphological comparison of *Lithocarpus dahouaiensis* with *Lithocarpus macphailii* and *Lithocarpus encleisocarpus* is also provided.

Key words

Da Huoai, Fagaceae, Lam Dong Province, *Lithocarpus*, *Lithocarpus dahuoaiensis,* Vietnam

Introduction

Lithocarpus Blume is the second largest genus of the family Fagaceae, comprising 341 species (The Plant List 2013). The genus is commonly known as Stone Oaks and widely distributed throughout the tropical and sub-tropical broad-leaved evergreen forests in East and Southeast Asia, extending to New Guinea (Cannon 2001, Phengklai 2008). In North America, one species of *Lithocarpus, L. densiflorus* (Hook. & Arn.) had been known, but has recently been treated as a member of a new monotypic genus *Notholithocarpus* (Manos *et al.* 2008). The center of diversity is in East to Southeast Asia, where 123 species

are enumerated in China (Huang *et al.* 1999), 58 species in Thailand (Phengklai 2008, Strijk *et al*. 2014) and 115 species in Vietnam (Ban 2005, Ho 2003).

In Vietnam, the species of Fagaceae are highly diversified and can be seen in various forest types, from dry evergreen forest at lowland to montane evergreen forest at high mountains. A total of 216 species and two varieties in six genera have been recorded in the country (Ho 1999, Ban 2005, Linh *et al.* 2013, Vuong and Xia 2014), which represents 66% of the total world genera and 24% of the total world species diversity in this family. One species of *Fagus* L., two species of *Castanea* Mill, 54 species of *Castanopsis* (D. Don) Spach., 43 species of *Quercus* L., one species of *Trigonobalanus* Forman and 115 species with two varieties of *Lithocarpus* have been found, indicating that *Lithocarpus* is the largest and most diversified genus of the family in Vietnam. Recently, several taxonomic works on Fagaceae of Vietnam were published (Deng et al. 2010; Linh *et al.* 2013, Vuong and Xia 2014), indicating that taxonomic studies of the family Fagaceae in Vietnam are still required.

Lam Dong Province is located in Central highland of Vietnam (Fig. 1.1) and has long been known as one of the biodiversity hotspots in Vietnam. In June 2015, the International Coordinating Council of UNESCO's Man and the Biosphere Program added 20 new sites to the World Network of Biosphere Reserves, among which Langbiang biosphere reserve in Lam Dong Province was one of the sites selected (UNESCO 2015). In the region, 3,490 species of vascular plants have been recorded, including 131 and 45 threatened species which are listed in Vietnam's Red Book and IUCN Red List Categories, respectively (Ban *et al*. 2007, IUCN 2012). As for Fagaceae, 90 species, including 30 species of *Lithocarpus*, are recorded from Lam Dong Province (Ho 2003, Ban 2005, Dung 2007).

During our floristic inventory in Lam Dong Province in 2015, we discovered

several individuals resembling species of the genus *Lithocarpus*. Further study revealed that these did not resemble any species described previously. Here, it is described and illustrated as *Lithocarpus dahuoaiensis* Ngoc & L. V. Dung, sp. nov.

Materials and methods

The new species was discovered through literature review, as well as undertook a thorough examination of specimens in the herbaria at ANDA, BKF, DLU, FU, HN, K, KYO, L, P, VNM and digital images of specimens on JSTOR Global Plants, Herbier National de Paris, Muséum National d'Histoire Naturelle (P).

Taxonomy

Lithocarpus dahuoaiensis Ngoc & L. V. Dung, **sp. nov.**

Figs. 1.2, 1.3

Diagnosis. *Lithocarpus dahouaiensis* is morphologically similar to *Lithocarpus macphailii* (M.R.Hend.) Barnett and *Lithocarpus encleisocarpus* (Korth.) A. Camus in having completely entire leaf margin, solitary cupule, long stalks of fruits, deeply cup-shaped or turbinate cupules with the number of horizontal filiform lines. But *L. duhouaiensis* is distinct by its cupules enclosing ca. 1/2–2/3 of the nuts (vs. cupules almost completely covering the nut in *L. macphailii* and *L. encleisocarpus*), surface of the cupule densely tomentose inside and subtle hairy to very subtle hairy outside (vs. outside densely fulvous tomentose in *L. macphailii* and outside densely fulvous tomentose by stellate hairs in *L. encleisocarpus*), leaf blades glabrous adaxially, undersides covered with very short soft hairs and subtle (vs. densely glaucous tomentose with adpressed, stellate hairs abaxially in *L. macphailii,* pubescent then glabrescent abaxially in *L. encleisocarpus*), secondary veins 11–12 pairs (vs. 12–16 pairs in *L. macphailii* and 8–10 pairs in *L. encleisocarpus)* (Table 1.1).

Type. VIETNAM. Lam Dong Province, Da Huoai, along the 20 National Highway, in the lowland evergreen forest, alt. 225 m, 11°23'32.5" N, 107°33'56.3"' E, 14 June 2015, *N. Nguyen, D. Luong, B. Hoang, T. Nguyen. V3194* (holotype: KYO!; isotype: DLU!, FU!, HN!, K!, P!, VNM!).

Description. Evergreen tree, up to 35 m tall; young branchlets pubescent with white hairs, soon glabrous, greyish green *in vivo* and blackish brown *in sicco*; terminal buds ca. 10–12 mm long, bud scale 4–6 mm long, densely covered with whitish hairs. Stipules not seen. Leaves alternate, blades broadly elliptic to slightly obovate, ca. 15–27 × 6–11 cm, thickly coriaceous, base cuneate, margin entire, slightly recurved, apex acuminate or caudate, acumen ca. 5–10 mm long, glabrous adaxially, subtle short soft hairs abaxially; midrib slightly raised above, distinctly raised below glabrous, greenish yellow *in vivo*, reddish brown *in sicco*; secondary veins 11–12 pairs, clearly visible on both sides, flat to slightly prominent adaxially, prominent abaxially, veins curving smoothly and disappearing near margins, at an angle of 55–65 degree from the midrib, tertiary veins scalariform, invisible to faintly visible on both surfaces; petioles ca. 10–15 mm long, rounded, thickened, pubescent when young, glabrescent later. Flowers not seen. Infructescences erect, woody, 25 cm long, rachis densely adpressed hairy. Acorn solitary, ovoid or turbinate, 13–15 mm in height, 20–23 mm in diam. (including cupule); fruiting stalk 3–5 mm long, densely fulvous tomentose hair; Cupules, turbinate, base a little broader than the upper part, densely tomentose inside and invisible or subtle hairy outside, lamellate, wall woody, sometimes crackled, enclosing ca. 1/2–2/3 of the nut, 19–22 mm in diam., 12–14 mm in height; bractlets triangular, obscure, forming 6–7 dimly concentric flanges. Mature nut 20–23 mm in diam., 19–22 mm in height, densely white tomentose; scar created by cupule at the base is deeply concave, ca. 13–15 mm in diam.; wall woody, crackled; apex abruptly acuminate, ca. 1.5–2 mm in height.

Phenology. Mature fruits were collected in June.

Distribution and habitat. Vietnam (so far known from Lam Dong Province and Dong Nai Province split by a boundary along National highway 20). (Figure 1.1)

Etymology. The specific epithet is derived from the type locality, Da Huoai, Lam Dong Province, Central Highland Vietnam.

Conservation status. Data Deficient (DD). Three fruiting individuals were found at the type locality, along the Chuoi pass of the 20 National highway. In addition, a staff of Dong Nai Culture and Nature Reserve had collected this species at Ma Da, Vinh Cuu, Dong Nai Province, indicating its wide distribution around the type locality. However, at present we have no reliable information on its population size. Further investigations are needed to determine the conservation status and actual population size in its natural habitat.

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Legends

Table 1.1: Morphological comparison between *Lithocarpus dahouaiensis* Ngoc & L.V. Dung, sp. nov. with *Lithocarpus macphailii* (M.R.Hend.) Barnett and *Lithocarpus encleisocarpus* (Korth.) A.Camus.

Characters	***L. dahouaiensis***	***L. macphailii***	***L. encleisocarpus***
Leaf margin	Entire	Entire	Entire
Leaf surface	Glabrous above, very short soft hairs and subtle beneath.	Densely glaucous tomentose with adpressed, stellate hair on lower surface	Subglabrous on upper surface, densely glaucous adpressed stellate-hairy on lower surfaces
Leaf size (cm)	15–27 × 6–11	15–22 × 6–8	12–15 × 4–6
Length of petioles	10–15 mm long	10–17 mm long	5–15 mm long
Number of secondary veins	11–12 pairs	12–16 pairs	(7–)8–10(–12) pairs
Length of fruit stalk	3–5 mm long	Up to 5 mm long	10–15 mm long
Acorn size (in diam.)	20–23 mm	20–25 mm	20–27 mm
Cupule size	12–14 mm high × 20–23 mm across	7–15 mm high × 20–30 mm across	N/A
Cupule outside	Faintly or very faintly visible hairs	Densely fulvous-tomentose	Densely fulvous tomentose by stellate hairs
Horizontal rings in cupule	6–7, dimly concentric flanges	5–8, thin, more or less concentric	5–7, more or less concentric
Nut enclosure	Enclosing ca. 1/2–2/3 of the nut	Almost completely covering the nut	Completely enclosing the nut
Infructescence length	15–25 cm long	10–25 cm long	8–20 cm long

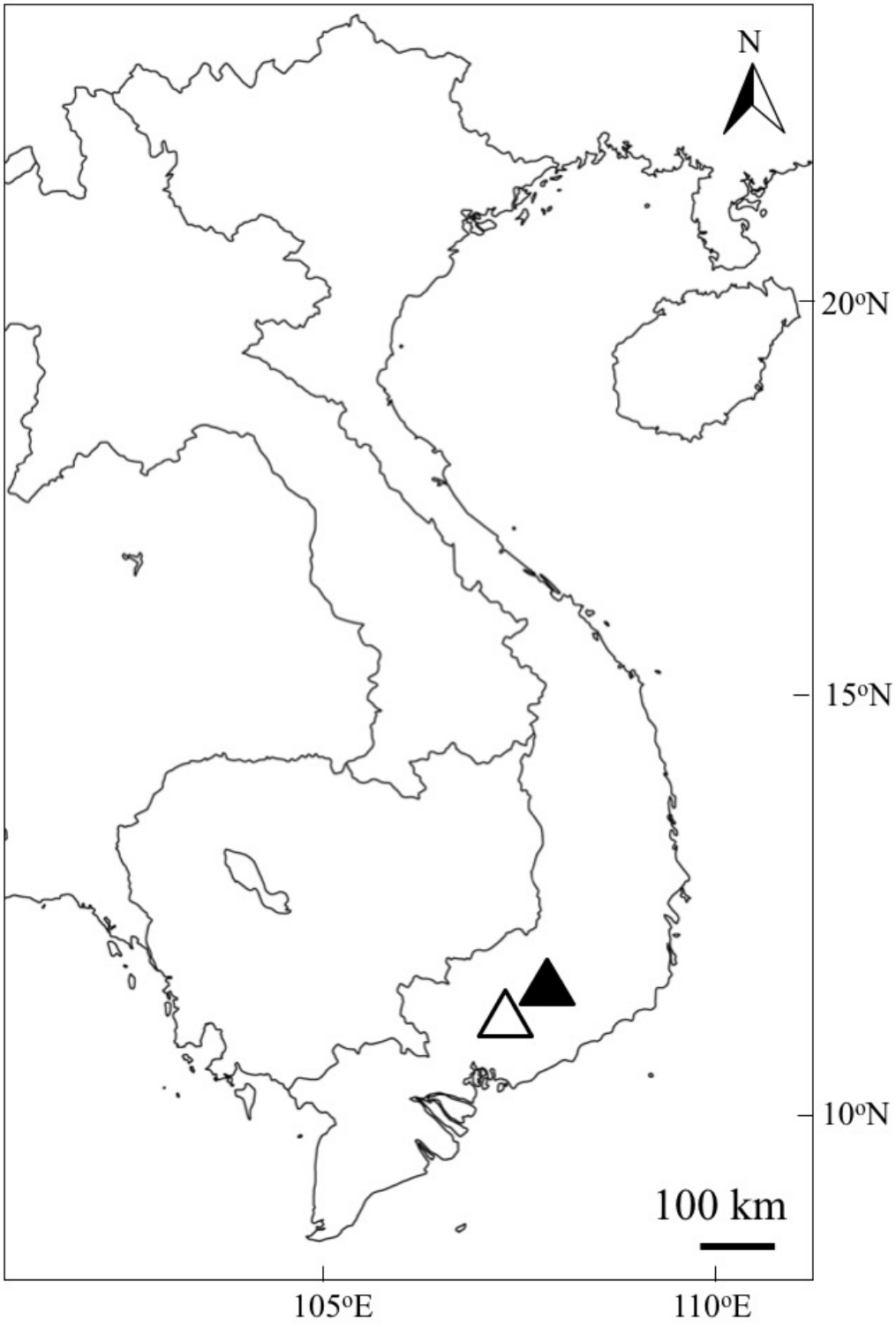

Figure 1.1: Distribution map of *Lithocarpus dahouaiensis Ngoc & Dung*. Black triangle, Da Houai, Lam Dong Province (Type locality); White triangle, Dong Nai Culture and Nature Reserve, Dong Nai Province.

Figure 1.2: *Lithocarpus dahouaiensis* Ngoc & Dung: **(A)** Leafy twig, **(B)** Buds, **(C)** Petiole, **(D)** Abaxial surface of mature Leaf, **(E)** Infructescence, **(F)** Mature fruit, **(G)** Cupule (left) and bottom of nut (right), **(H)** Vertical sections of nut.

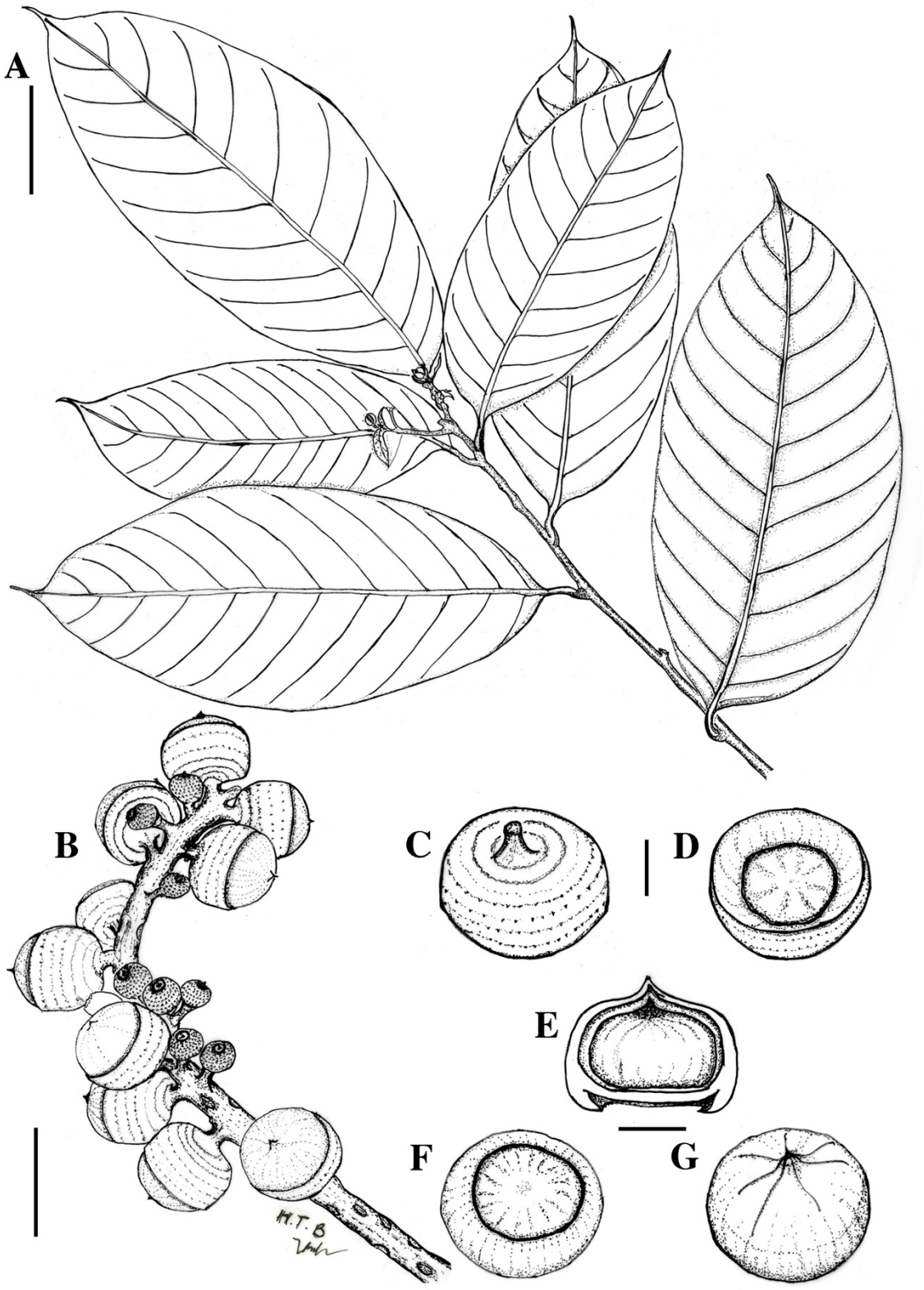

Figure 1.3: Line drawing of *Lithocarpus dahouaiensis* Ngoc & Dung: (**A**) Leafy twig, (**B**) Infructescence, (**C**) & (**D**) Cupule, (**E**) Vertical section of mature nut, (**F**) & (**G**) Mature nut. Scale bars (**A**) & (**B**) = 5 cm; (**C**)-(**G**) = 10 mm.

Chapter II

Two new species of *Lithocarpus* (Fagaceae) from Bidoup-Nui Ba National Park, Vietnam.

Abstract

Two new species, *L. bidoupensis* and *Lithocarpus hongiaoensis* are described and illustrated from Bidoup-Nui Ba National Park, Central Highland of Vietnam. Phylogeny based on multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) supports the distinction of those species from the previously known taxa in the region. The two new species are considered to be endemic to the Bidoup-Nui Ba national park and qualified as Critically Endangered for their conservation status.

Key words

DNA barcoding, Fagales, Indochina, taxonomy

Introduction

The family Fagaceae Dumortier (1829: 11) is highly diversified in Vietnam and 216 species of 6 genera have been reported in various forest types, from dry evergreen forest at lowlands to montane evergreen forest in the higher elevation (Ban 2003, Ho 2003, Ngoc *et al*. 2016). Recently, three species of Fagaceae were newly described from Vietnam: *Castanopsis grandicicatrica* Vuong & Xia (2014: 31) *Castanopsis multiporcata* Vuong & Xia (2014: 34) and *Lithocarpus dahuoaiensis* Ngoc *et al.* (2016: 25).

Lithocarpus Blume (1826: 526) is the largest and most diversified genus of the family in Vietnam, including 118 species and two varieties, among which 44 species are endemic (Ban 2003, Ho 2003, Linh *et al*. 2013, Ngoc *et al*. 2016). However, we often

encounter species of *Lithocarpus* that are difficult to be identified at species level, indicating we need more efforts for clarifying its diversity and taxonomy accurately.

Bidoup-Nui Ba National Park is the core zone of the Langbiang biosphere reserve, which is located in Lam Dong Province on Central highland of Vietnam. The national park of ca. 70,038.45 ha covers almost the entire Langbian Plateau (Bidoup-Nui Ba National Park 2016), harboring 1933 species of vascular plants (Bidoup-Nui Ba National Park 2016) including 62 threatened species (Ban *et al*. 2007, IUCN 2012), and 29 endemic species (Bidoup-Nui Ba National Park 2016, Tagane *et al.* 2017). For Fagaceae, 25 species of *Lithocarpus,* nine species of *Castanopsis* (Don 1825: 56) Spach (1841: 185), nine species of *Quercus* Linnaeus (1753: 994), and a species of *Trigonobalanus* Forman (1962: 140) have been recorded from Bidoup-Nui Ba National Park (Dung 2005, Ngoc *et al*. 2016).

During our floristic research in Bidoup-Nui Ba National Park (Fig. 2.1) from 2015–2016, we found two undescribed species of the genus *Lithocarpus*. We here describe and illustrate them as *Lithocarpus bidoupensis* Ngoc & Tagane, sp. nov and *Lithocarpus hongiaoensis* Ngoc & Binh, sp. nov., based on comparisons of morphology and phylogeny based on genome-wide short DNA sequences determined using the next generation sequencing platform (MIG-seq; Suyama and Matsuki 2015) with related species.

Materials and methods

Taxon Sampling

In the present study, we conducted botanical inventories in Bidoup-Nui Ba National Park and surroundings areas and collected 18 samples of the genus *Lithocarpus* including seven described species: *L. coalitus* (Hickel & Camus, 1923: 606) Camus (1931: 39) (voucher specimen no. *V4191*), *L. dahuoaiensis* (*V3194*) *L. giantophyllus* (Hickel & Camus, 1921: 398) Camus (1931: 40) (*V3185*), *L. lemeeanus* Camus (1943: 84) (*V4273*),

L. licentii Camus (1942: 359) (*V3205*), *L. pseudomagneinii* Camus (1942: 360) (*V3223*), *L. stenopus* (Hickel & Camus, 1928: 365) Camus (1931: 42) (*V3187*), and two undescribed species *V4320* and *V3235*, hereafter we named as *Lithocarpus bidoupensis* Ngoc & Tagane, sp. nov. and *Lithocarpus hongiaoensis* Ngoc & Binh, sp. nov., respectively. In addition, the species having similar morphological characteristics to *L. bidoupensis* and *L. hongiaoensis* were collected from the other areas in Vietnam: *L. hancei* (Bentham, 1861: 322) Rehder (1919: 127) (*V4800, V4924* & *V5111*) from Hoang Lien National Park, *L. longipedicellatus* (Hickel & Camus, 1928: 365) Camus (1931: 41) (*V3813*) and *L. vinhensis* Camus (1948: 112) (*V3787*) from Vu Quang National Park, *L. ombrophilus* Camus (1945: 82) (*V3000*) from Bach Ma National Park, *L. ochrocarpus* Camus (1938: 182) (*V3115*) from Ba Na Nature Reserve, *L. aggregatus* Barnett (1938: 104) (*V6288*) from Ngoc Linh Nature Reserve and *L. dahuoaiensis* (*V5404*) from Dong Nai Culture and Nature Reserve. Two species of *Castanopsis*: *C. cerebrina* (Hickel & A.Camus 1921: 408) Barnett (1944: 183), *C. piriformis* Hickel & A.Camus (1921 publ. 1922: 395), three species of *Quercus*: *Q. helferiana* A.DC. (1864: 101), *Q. poilane* Hickel & A.Camus (1921: 384), *Q. langbianensis* Hickel & A.Camus (1921: 382) and *T. verticillata* Forman (1962: 140) were included in the phylogenetic analysis as outgroups (Fig. 2.1, Supplementary 2.S1).

Morphological observations

We compared morphological traits of the unknown species with those of related species using taxonomic literature (Bentham 1861, Camus 1938 & 1943, Chun 1947, Hu 1951, Soepadmo 1972, Huang *et al.* 1999, Ban 2003, Ho 2003, Phengklai 2008), specimens kept in the herbaria ANDA, BKF, DLU, HN, KYO, P, and VNM, and digitized plant specimen images available on the web of JSTOR Global Plants (https://plants.jstor.org/) and Chinese Virtual Herbarium (http://www.cvh.org.cn/).

DNA extraction and MIG-seq analysis

Leaf pieces were dried using silica-gel in the field and DNA was isolated by the CTAB method (Doyle & Doyle 1987) with minor modifications described in Toyama *et al*. (2015). The DNA extracted from 18 taxa of *Lithocarpus* including two new species and six taxa of outgroup were diluted to *ca*. 10 ng/μl and used as templates to amplify thousands of short sequences (loci) from a wide variety of genomes with a standard PCR protocols according Suyama and Matsuki (2015). MIG-seq library was constructed as described in Suyama and Matsuki (2015) with a minor update by using dual-indexed primers. The 1st PCR, multiple non-repetitive regions from various inter-simple-sequence repeats (ISSRs) were amplified from genomic DNA by multiplexed PCR with tailed ISSR forward and reverse primers sets: $(ACT)_4TG$, $(CTA)_4TG$, $(TTG)_4AC$, $(GTT)_4CC$, $(GTT)_4TC$, $(GTG)_4AC$, $(GT)_6TC$, and $(TG)_6AC$. The first PCR product were diluted and used as the templates for the 2nd PCR (tailed PCR). Then, 3 μl of each 2nd PCR product was pooled in equimolar concentrations as single mixture library. The mixture was then purified and the size range of 350–800 bp were isolated by a Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA). Quantitative PCR was performed to measure final concentration of size-selected library with approximately 10 pM and then used for sequencing on an Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 cycle, Illumina).

Fastx-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and TagDust program (Lassmann et al. 2009) were used to remove the primer regions and control quality of the raw data from 24 samples following Suyama and Matsuki (2015). Stacks software package version 1.35 (Catchen et al. 2011) is then used to assemble loci from the quality-filtered reads data with the *de novo* map pipelines (ustacks, cstacks, sstacks). Later, a table of presence/absence of loci in each individual was prepared after the population pipeline with parameters setting as described by Binh *et al*. (2018). The presence/absence (1/0) data of loci were used to infer the phylogenetic tree using PHYLYP ver. 3.695 (Felsenstein 2005)

as follows; Fist we used Seqboot for 1000 times resampling from presence/absence data of loci. Second, distance matrices were computed with Restdist program. Third, the distance matrices are used to infer the phylogenetic trees with Neighbor program. Finally, a consensus tree was constructed with Consense. The software FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) was used to visualized resulted tree.

Results

Morphological observation

Two unknown species of *Lithocarpus* were not assignable to any of the species recognized in Vietnam and its surrounding countries. According to the key in the Flora of China (Huang *et al*. 1999), *L. bidoupensis* is most similar to *L. hancei* in having leaf blade not very narrow, adaxially glabrous, green and shining on both surfaces, margin entire, base neither auriculate nor rounded, secondary veins 10 or more, cupules in clusters of 3 along rachis, sessile and enclosing almost 1/3 of nut, cupule bracts not linear, nut glabrous and nut scar concave. *Lithocarpus hongiaoensis* corresponds to *L. elmerrillii* Chun (1947: 232) in having leaf margin entire, cupules not completely enclosing, almost sessile pedicel, cupule scales imbricate, nut glabrous with basal scar concave. From Vietnam, species most consistent with the above diagnostic features of *L. bidoupensis* is *L. licentii*, whereas species having the above diagnostic features of *L. hongiaoensis* are *L. lemeeanus* and *L. ochrocarpus*. Thus, we morphologically compared *L. bidoupensis* with *L. hancei* and *L. licentii,* and *L. hongiaoensis* with *L. elmerrillii*, *L. lemeeanus* and *L. ochrocarpus*.

After the examination of the morphology among these species, *L. bidoupensis* is distinguished from *L. hancei* by its much shorter petioles, longer infructescences, bigger cupules and larger scar of the nut. Also, *L. bidoupensis* is distinguished from *L. licentii* in having shorter petioles, fewer number of secondary veins, much shorter infructescences,

clustered cupule (vs. solitary), cupule covering less than 1/3 of the nut (vs. 1/2–2/3 of the nut in *L. licentii*), and concave basal scar (Table 2.1).

Lithocarpus hongiaoensis differs from *L. elemerrilii* of China in having longer petioles and infructescences, smaller nuts and the ratio of cupule/nut height more than 1.5 (vs. ca. 0.4). *Lithocarpus hongiaoensis* is distinct from both *L. lemeeanus* and *L. ochrocarpus* in having longer petioles and infructescences, cupule solitary (vs. 3 cupules clustered), covering less than 1/2 of the nut, and the ratio of cupule/nut height more than 1.5 (vs. ca. 0.9 and 1.1 in *L. lemeeanus* and *L. ochrocarpus*, respectively) (Table 2.2).

A phylogenetic tree using MIG-seq

In the NJ consensus tree based on presence/absence data of 24,731 MIG-seq loci (Fig. 2.2), except outgroup, two sister clades, Clade 1 and Clade 2, were supported by bootstrap value 77 and 100%, respectively. While *L. hancei,* a species is morphologically similar to *L. bidoupensis* was clustered with *L. gigantophyllus* in Clade 1, *L. bidoupensis* was placed in Clade 2c. *L. hongiaoensis* was sister to *L. ochrocarpus* and *L. coalitus* in Clade 2a, the monophyly of these species was supported by 80% bootstrap value. *Lithocarpus licentii*, another species morphologically similar to *L. bidoupensis*, was placed in Clade 2b and sister to *L. pseudomagneinei, L. lemeeanus* and *L. dahuoaiensis* with 100% bootstrap value. *Lithocarpus bidoupensis* was clustered with three other species *L. longipedicelatus, L. vinhensis,* and *L. ombrophilus* and was supported by 94 % bootstrap value, and then these species were sister to two other species *L. stenopus* and *L. aggregatus* in Clade 2c.

Discussion

The morphological comparison and phylogenetic analysis provided evidence of the validity of two new species. *Lithocarpus bidoupensis* is most similar to *L. hancei* that was collected in Hoang Lien National Park, and also similar to *L. licentii* that occurred in the same locality with *L. bidoupensis*. However, the new species is clearly different from both in many traits (Table 2.1). The molecular phylogenetic tree also supported this disjunction in that *L. bidoupensis* was sister to neither of *L. hancei* and *L. licentii*. Among Vietnamese species, *L. hongiaoensis* is most similar to *L. lemeeanus* and *L. ochrocarpus,* of which the latter showed the sister relationship to *L. hongiaoensis* in the molecular phylogenetic tree. However, *L. hongiaoensis* is distinct from *L. ochrocarpus* in solitary cupule having a distinct stalk and covering 1/3–1/2 of nut (Table 2.3). We collected both *L. hongiaoensis* and *L. lemeeanus* in Bidoup-Nui Ba National Park, but the two species differed in many traits summarized in Table 2.2. We need further molecular phylogenetic studies to clarify the relationship between *L. hongiaoensis* and a Chinese species *L. elmerrillii*. However, morphological differences are distinct enough to distinguish them as different species (Table 2.2).

Taxonomic treatments

Lithocarpus bidoupensis Ngoc & Tagane, *sp. nov.* (Figs. 2.3 & 2.4).

Type:—VIETNAM. Lam Dong Province: Bidoup-Nui Ba National Park, in hill evergreen forest dominated by the species of Fagaceae, 1,698 m elev., 12°09′52.95″N, 108°32′00.38″E, 24 February 2016, *Tagane S., Toyama H., Nagamasu H., Naiki A., Dang V.S., Nguyen V.N. & Wai J. V4320* (holotype KYO!, isotypes DLU!, FU!, HN!, K!, P!, VNM!).

Diagnosis:—*Lithocarpus bidoupensis* is similar to *L. hancei* but differs mainly in its shorter petioles, much bigger cupules and larger basal scar of the nut. The new species is distinguished from *L. licentii* in having shorter petioles, fewer number of secondary

veins, much shorter infructescences, clustered cupule, cupule covering less than 1/3 of the nut, concave basal scar.

Description:—Evergreen tree, up to 27 m tall. Branches yellowish green when young, turning greyish brown when old, glabrous, sparsely lenticellate. Terminal and lateral buds ovoid, up to 7 mm long. Leaves alternate; petiole 3–5 mm long, glabrous; blade elliptic to oblong-elliptic, obovate-elliptic, 6–11.6 × 3.1–5.1 cm, coriaceous, apex acuminate or attenuate, acumen up to 0.9 cm long, base cuneate, rarely obtuse, margin completely entire, glabrous, glossy green on both surfaces; midrib prominent on both surfaces, secondary veins 10–12 pairs, prominent abaxially, at an angle of 55–70° from the midrib; tertiary veins scalariform-reticulate, visible abaxially. Inflorescences not seen. Infructescences a woody spike, 8.4–11.5 cm long, rachis 0.6–0.8 cm thick. Cupules usually in cluster of 3, sessile to 6 mm stalked, fused or adnate at the base each other, depressed obconical or saucer-shaped, 0.8–1.4 cm high, 2.3–2.8 cm in diam., enclosing 1/3 of the nut, pubescent with short grayish indumentum outside; wall woody, ca. 2 mm thick, with brown triangular scales outside, the scales up to 4 × 4 mm, imbricated scales or arranged in 3 or 4 interrupted concentric rings. Nut broadly ovoid-conical to depressed ovoid-globose, 1.5–1.6 cm high, 2.1–2.3 cm in diam., glabrous, brown to blackish brown; basal scar slightly concave, 1.4–1.9 cm in diam.

Phenology:—Unknown. Fallen fruits were collected in February.

Distribution:—Vietnam (so far known only from Bidoup-Nui Ba National Park, Lam Dong Province). (Fig. 2.1)

Etymology:—The specific epithet is derived from the type locality, Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam.

GenBank accession No.:—*Tagane et al. V4320*: LC318961 (*rbcL*) LC318547 (*matK*), KY940070 (ITS)

Conservation status:—Critically Endangered (CR). During our field surveys in the protected areas of Bidoup-Nui Ba National Park for 10 days from the elevation 1500–1850 m, only one individual of Lithocarpus bidoupensis with a number of fallen fruits was collected at 1,698 m alt., and later, numerous of last year fruits were also collected in another area inside the National Park. Based on criterion D of the IUCN Red List criteria (IUCN 2012), this species is qualified as CR.

Lithocarpus hongiaoensis Ngoc & Binh, *sp. nov.* (Figs. 2.5 & 2.6).

Type: VIETNAM. Lam Dong Province, Bidoup-Nui Ba National Park, edge of evergreen forest at roadside, 1,580 m elev., 12°10′35.9″N 108°42′25.1″E, 19 June 2015, *N. Nguyen, D. Luong, B. Hoang V3235* (holotype KYO!; isotypes DLU!, FU!, HN!, K!, P!, VNM!).

Diagnosis:—*Lithocarpus hongiaoensis* is similar to *L. elmerrillii* but distinct in having more longer petioles and infructescences, smaller nuts and the ratio of cupule height/nut height more than 1.5 (vs. ca. 0.4). The new species also similar to *L. lemeeanus* and *L. ochrocarpus* but differs in having longer petioles and infructescences, cupule solitary and covering less than 1/2 of the nut, the ratio of cupule /nut height more than 1.5 (vs. ca. 0.9 and 1.1 in *L. lemeeanus* and *L. ochrocarpus*, respectively).

Description:—Evergreen tree, up to 25 m tall. Twigs blackish gray, glabrescent, densely lenticellate. Stipules narrowly triangular, ca. 5 × 1 mm, covered with dense indumentum outside, almost glabrous inside. Leaves alternate; petiole 2.1–3 cm long, glabrous; blade narrowly elliptic to lanceolate, 9.6–14.5 × 2.5–3.8 cm, coriaceous, apex acuminate with acumen up to 1.5 cm long, base attenuate and decurrent on petiole, margin entire, glabrous adaxially, adherent waxy scale abaxially; midrib flat or slightly prominent near base

adaxially, prominent abaxially, greenish yellow *in vivo*, reddish brown *in sicco*; secondary veins 8–11 pairs, prominent abaxially, at an angle of 35–45° from the midrib, tertiary veins scalariform, faintly visible or invisible. Young inflorescences terminal, ca. 5–7 cm long. Infructescences terminal, erect, 12.5–16.5 cm long, 0.4–0.6 cm thick at base, grayish brown, lenticellate, covered with indumentum. Cupules solitary, sessile to 2 mm stalked, obconical to saucer-shaped, 1.2 cm high, 2.1 cm in diam., enclosing 1/3–1/2 of the nut; wall woody, ca. 2 mm thick, with triangular scales not united into concentric rings; the scales up to 4 mm long, apex shortly acuminate, covered with dense grayish indumentum. Nut strongly depressed ovoid, 0.6–0.8 cm high, 1.2–1.5 cm in diam., glabrous, reddish brown to grayish brown; basal scar slightly concave, ca. 1.4 cm in diam.

Phenology:—Mature fruits were collected in June.

Distribution:—Vietnam (so far known only from Mt. Hon Giao of Bidoup-Nui Ba National Park, Lam Dong Province). (Fig. 2.1)

Etymology:—The specific epithet is derived from the type locality, Mt. Hon Giao of Bidoup-Nui Ba National Park, Lam Dong Province, Vietnam.

GenBank accession No.:—*Ngoc et al. V3235*: LC318956 (rbcL) LC318542 (*matK*), KY851759 (ITS)

Conservation status:—We found only three individuals of *Lithocarpus hongiaoensis* along the road inside the protected area of Bidoup-Nui Ba National Park. According to the criterion D of the IUCN Red List criteria (IUCN 2012), this species is qualified as CR.

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Legends

SUPPLEMENTARY 2.S1. List of taxa sampled in this study with vouchers and GenBank accession number.

Species	Vouchers	Localities	GenBank accession number		
			rbcL	***matK***	**ITS**
L. aggregatus	*Tagane et al. V6288* (DLU, FU)	Ngoc Linh NR	LC318967	LC318550	MF770309
C. cerebrina	*Ngoc et al. V5503* (DLU, FU)	Pumat NP	N/A	N/A	N/A
C. piriformis	*Tagane et al. V6689* (DLU, FU)	Ngoc Linh NP	N/A	N/A	N/A
L. bidoupensis	*Tagane et al. V4320* (DLU, FU, VNM)	Bidoup-Nui Ba NP	LC318961	LC318547	KY940070
L. coalitus	*Tagane et al. V4191* (DLU, FU, VNM)	Bidoup-Nui Ba NP	LC318959	LC318545	MF770305
L. dahuoaiensis	*Ngoc et al. V3194* (DLU, FU, HN, K, KYO, P, VNM)	Bidoup-Nui Ba NP	LC318953	LC318551	KY436002
	Ngoc et al. V5404 (DLU, FU)	Dong Nai CNR	LC318964	LC318548	MF770307
L. gigantophyllus	*Ngoc et al. V3185* (DLU, FU)	Bidoup-Nui Ba NP	LC318951	LC318538	MF770299
L. hancei	*Ngoc et al. V5111* (DLU, FU)	Hoang Lien NP	LC318963	LC318970	MF952868
	Ngoc et al. V4800 (DLU, FU)	Hoang Lien NP	LC318962	LC318968	MF770304
	Ngoc et al. V4924 (DLU, FU)	Hoang Lien NP	LC321977	LC318969	MF952867
L. hongiaoensis	*Ngoc et al. V3235* (DLU, FU)	Bidoup-Nui Ba NP	LC318956	LC318542	KY851759
L. lemeeanus	*Tagane et al. V4273* (DLU, FU)	Bidoup-Nui Ba NP	LC318960	LC318546	MF770306
L. licentii	*Ngoc et al. V3205* (DLU, FU)	Bidoup-Nui Ba NP	LC318954	LC318540	MF770301
L. longipedicellatus	*Nguyen et al. V3813* (DLU, FU)	Vu Quang NP	LC318958	LC318544	MF770304
L. ochrocarpus	*Tagane et al. V3115* (DLU, FU)	Ba Na NR	LC318950	LC318421	MF770298
L. ombrophilus	*Yahara et al. V3000* (DLU, FU)	Bach Ma NP	LC318949	LC318420	MF770297
L. pseudomagneinii	*Ngoc et al. V3223* (DLU, FU)	Bidoup-Nui Ba NP	LC318955	LC318541	MF770302
L. stenopus	*Ngoc et al. V3187* (DLU, FU)	Bidoup-Nui Ba NP	LC318952	LC318539	MF770300
L. vinhensis	*Nguyen et al. V3787* (DLU, FU)	Vu Quang NP	LC318957	LC318543	MF770303

Q. helferina[*]	*Ngoc et al. V3169* (DLU, FU)	Lam Dong	LC318781	LC318501	MF770279
Q. langbianensis[*]	*Tagane et al. V3962* (DLU, FU)	Bidoup-Nui Ba NP	LC318790	LC318510	MF770285
Q. poilanei[*]	*Yahara et al. V2986* (DLU, FU)	Bach Ma NP	LC318774	LC318494	MF770273
T. verticillata	*Yahara et al. V5764* (DLU, FU)	Vu Quang NP	LC318965	LC318549	MF770308

NP = National Park; NR = Nature Reserve; CNR = Culture and Nature Reserve; (*) From Binh *et al*. (2018).

TABLE 2.1. Morphological comparison of *Lithocarpus bidoupensis* with its relatives.

Characters	***L. bidoupensis***[3]	***L. hancei***[1,2,3]	***L. licentii***[1,2,3]
Leaf margin	Entire	Entire	Entire
Leaf surface	Glabrous, glossy green on both surfaces	Glabrous, green and shining on both sides	Glabrous
Leaf blade shape	Elliptic to oblong-elliptic or obovate-elliptic	Variable in shape: ovate, broadly elliptic, obovate-elliptic, narrowly elliptic, or lanceolate	Oblanceolate or ovate-lanceolate
Leaf blade size	6–11.6 × 3.1–5.1 cm	5–10 x 2.0–5 cm	8–16 x 3.5–7 cm
Petiole length	3–5 mm long	10–22 mm long	5–15 mm long
Number of Secondary veins	10–12 pairs	6–13 pairs	12–15 pairs
Infructescences length	8.4–11.5 cm long	6–8 cm long	15–20 cm long
Cupule	Clusters of 3, depressed obconical or saucer-shaped	Clusters of 3–5, shallowly bowl-shaped to plate-shaped	Solitary
Cupule size	0.9–1.3 cm high x 2.3–2.7 cm in diam.	0.3–0.7 cm high x 0.6–0.8 cm in diam.	1.2–1.4 cm high x 2.2–2.3 cm in diam.
Scales arrangement	Imbricate or arranged in 3 or 4 interrupted concentric rings	Imbricate and appressed or connate into a few concentric rings	Imbricate
Nut shape	Broadly ovoid-conical to depressed ovoid-globose	Depressed globose, subglobose, or broadly conical	Depressed
Nut size	1.5–1.6 cm high × 2.1–2.3 cm across	1.4–1.8 cm high × 0.6–1.0 cm across	1.2–1.3 cm high x 2–2.3 cm across
Nut enclosure by cupule	1/4–1/3 of the nut	1/3 of the nut	1/2–2/3 of the nut
Basal scar of the nut	1.4–1.7 cm in diam., slightly concave	0.5–1.0 cm in diam., concave	1.7–1.8 cm in diam., convex

[1]Derive from original description; [2]Derive from digitized type specimen image, [3]Derive from this study collection.

TABLE 2.2. Morphological comparison of *Lithocarpus hongiaoensis* Ngoc & Binh, sp. nov. with its relatives.

Characters	***L. hongiaoensis***[3]	***L. elmerrillii***[1,2]	***L. lemeeanus***[1,2,3]	***L. ochrocarpus***[1,2,3]
Leaf margin	Entire	Entire	Entire	Entire
Leaf surface	Glabrous upper, adherent waxy scale abaxially	Glabrous upper, adherent waxy scale abaxially	Glabrous upper, adherent waxy scale abaxially	Glabrous upper, adherent waxy scale abaxially
Leaf size	9.6–14.5 × 2.5–3.8 cm	7–15 × 2.5–6 cm	6–15 × 2.5–4.5 cm	9–11 × 2.5–4 cm
Petiole	2.1–3 cm long	1–1.3 cm long	0.8–1 cm long	1.2–2 cm long
Secondary veins	8–11 pairs	7–9 pairs	10–11 pairs	7–8 pairs
Infructescences length	12.5–16.5 cm long	Up to 7 cm long	8–12 cm long	8–10 cm long
Fruit-stalk	Sessile to ca. 2 mm long	Almost sessile	Almost sessile	Almost sessile
Cupule	Solitary, 1–1.2 cm high by 1.8–2.1 cm in diam.	Solitary, 0.8–1 cm high, by 1.5–2.4 cm in diam.	Clustered, 1.2–1.6 cm high, by 2–2.5 cm in diam.	Clustered, 0.7–0.8 cm high, by 0.8–1.2 cm in diam.
Nut size	0.6–0.8 cm high by 1.2–1.5 cm in diam.	1.8–2.4 cm high by 1.8 cm in diam.	1.5 cm high by 2 cm in diam.	0.6–0.7 cm high by 0.6–0.7 in diam.
Ratio of cupule height/nut height	More than 1.5	Ca. 0.4	Ca. 0.9	Ca. 1.1
Nut enclosure	Enclosing ca. 1/3–1/2 of the nut	Enclosing ca. 1/3 – 1/2 of the nut	More than 1/2 of the nut	Covering more than 1/2 of the nut
Scar of the nut	Slightly concave, 12–14 mm in diam.	Concave, 12–14 mm in diam.	Subconvex, 12–15 mm in diam.	N/A

[1]Derive from original description; [2]Derive from digitized type specimen image, [3]Derive from this study collection.

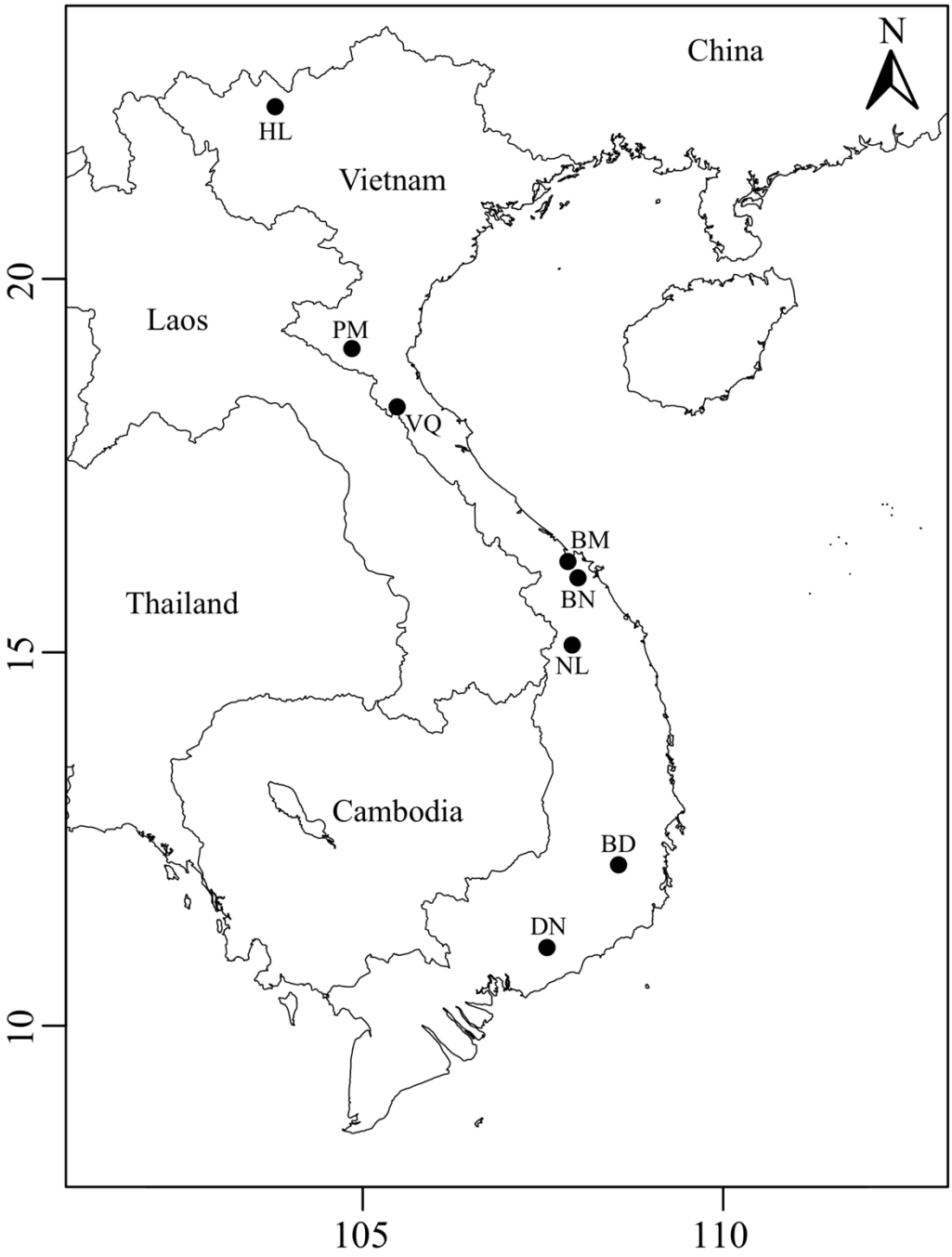

FIGURE 2.1. Collection sites in this study: (**HL**) Hoang Lien National Park, (**PM**) Pu Mat National P, (**VQ**) Vu Quang National Park, (**BM**) Bach Ma National Park, (**BN**) Ba Na Nature Reserve, (**NL**) Ngoc Linh Nature Reserve, (**BD**) Bidoup-Nui Ba National Park (type locality of *L. bidoupensis* and *L. hongiaoensis*), (**DN**) Dong Nai Culture and Nature Reserve.

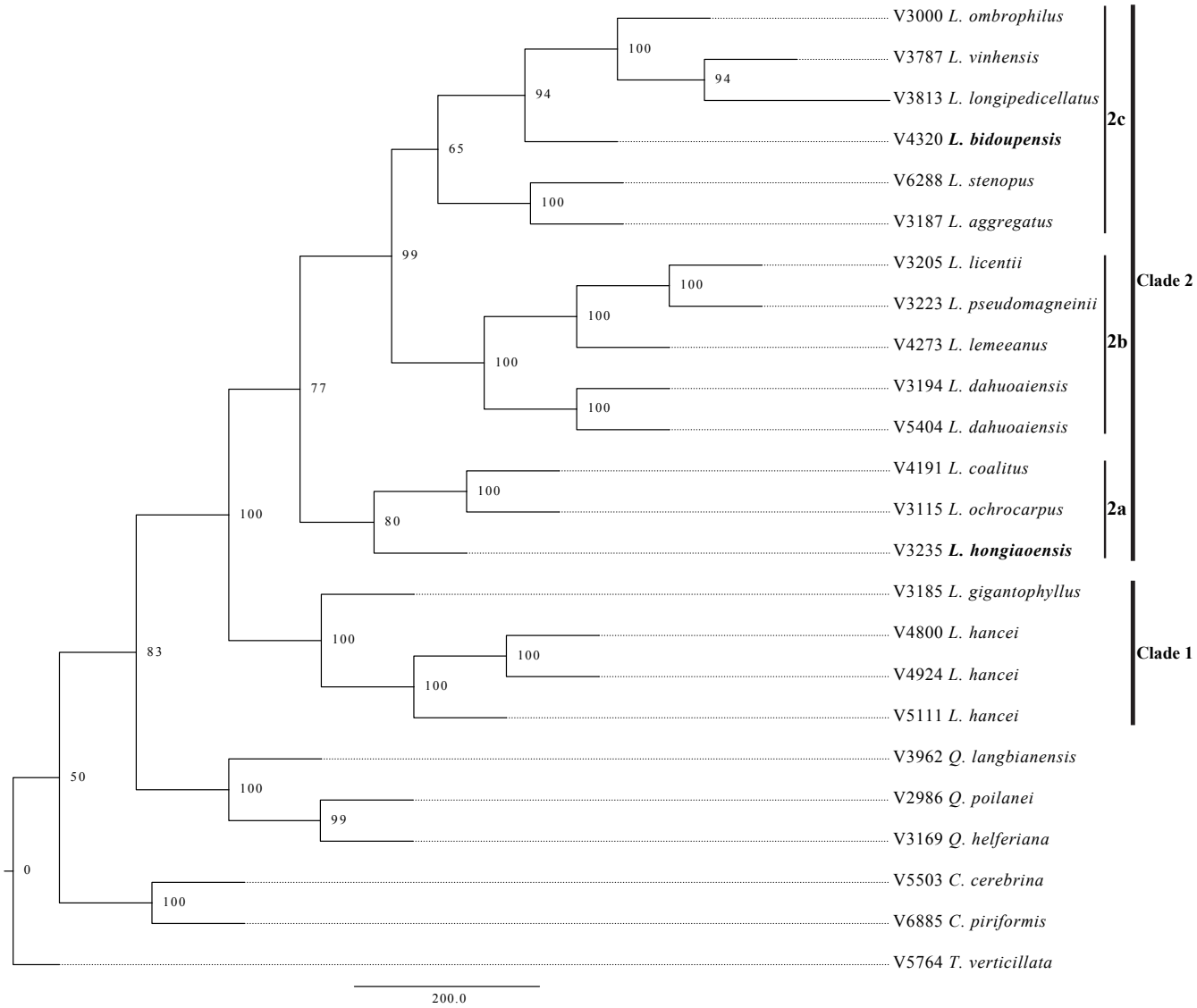

FIGURE 2.2. MIG_seq tree of 18 samples of *Lithocarpus* and outgroup based on presence/absence data of 24,731 MIG-seq loci. Branches are labeled with bootstrap support (% of 1000 replicates).

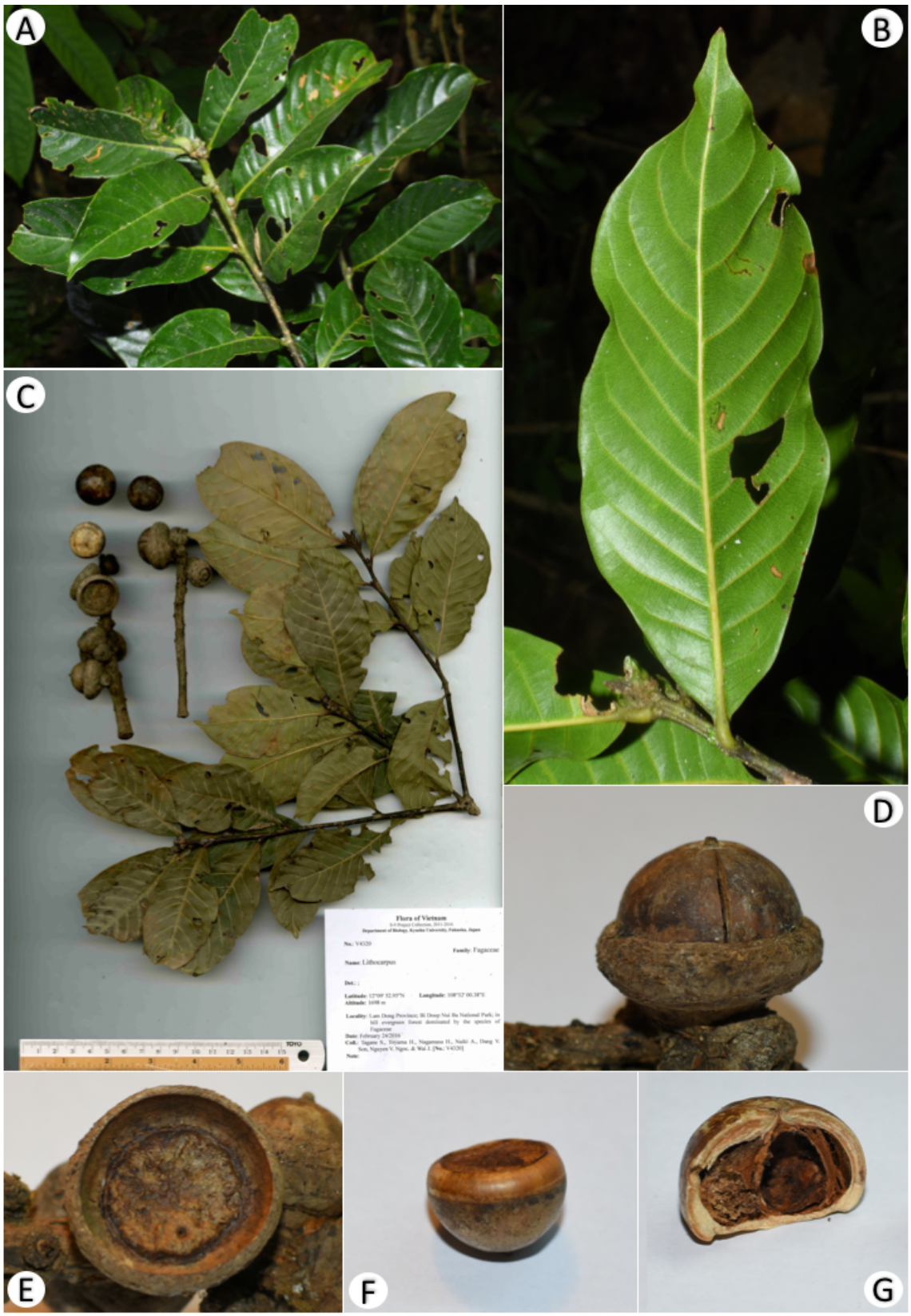

FIGURE 2.3. *Lithocarpus bidoupensis* Ngoc & Tagane: **A** Leafy twigs, **B** Abaxial leaf surface, **C** Holotype (*Tagane et al. V4320*, KYO), **D** Mature fruit, **E** Cupule, **F** Mature nut, **G** Section of mature nut.

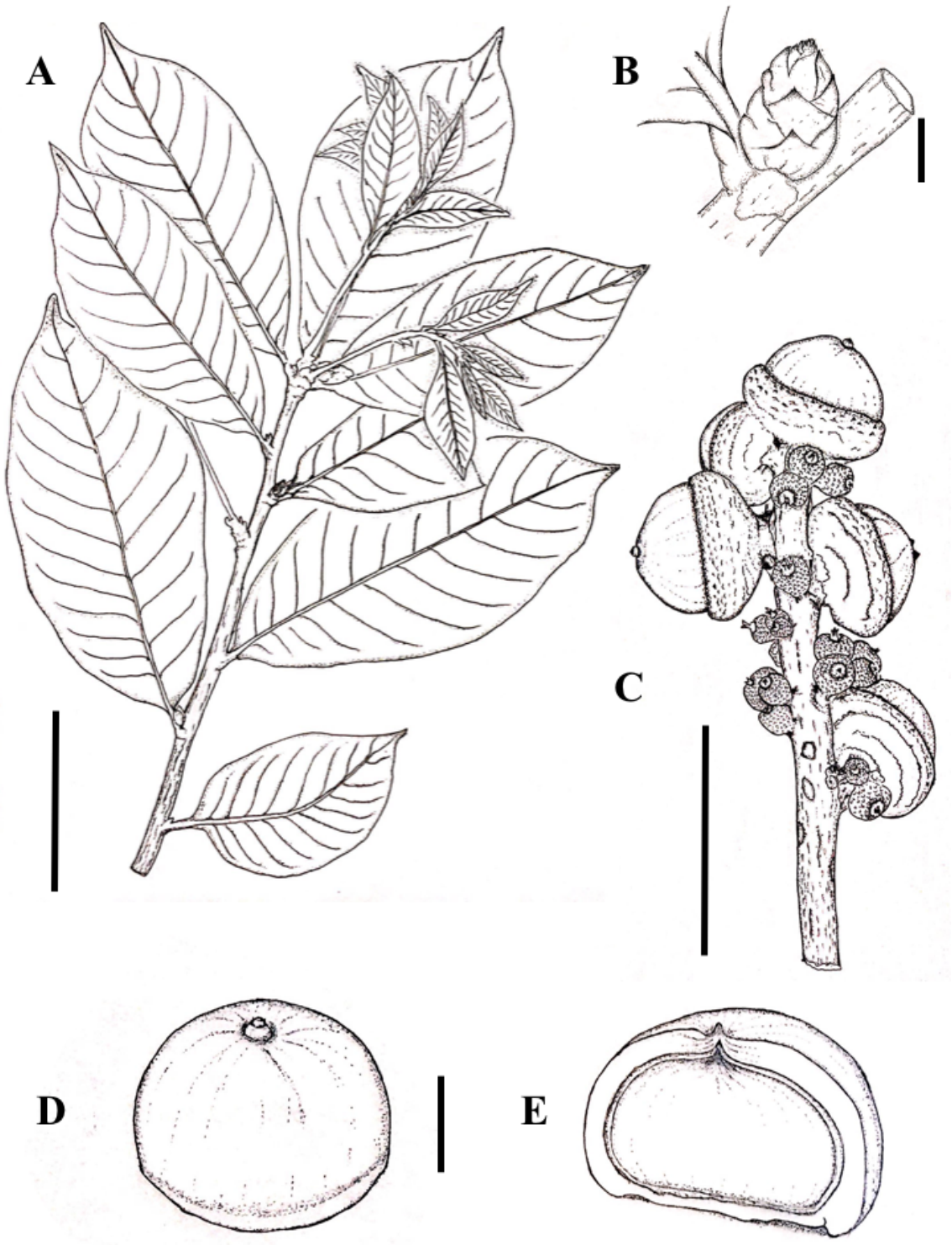

FIGURE 2.4. Illustrated of *Lithocarpus bidoupensis* Ngoc & Tagane: **A** Leafy twig, **B** Bud, **C** Infructescence, **D** Mature nut, **E** Vertical section of mature nut. Scale bars **A**, **C** = 5 cm; **B** = 3 mm, **D& E** = 1 cm. Drawn by Nguyen Van Ngoc & Hoang Thi Binh.

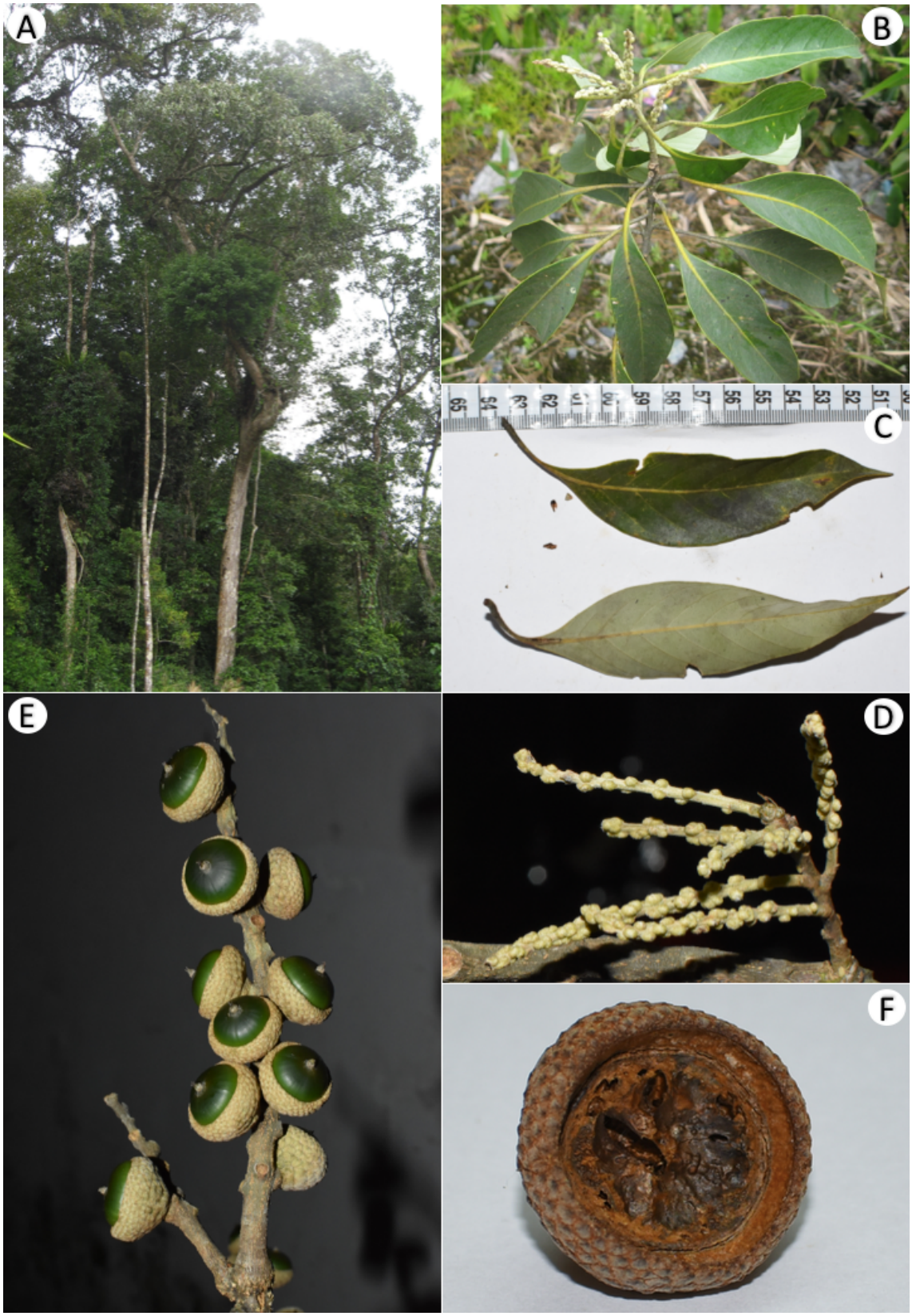

FIGURE 2.5. *Lithocarpus hongiaoensis* Ngoc & Binh: **A** Habit, **B** Twig with young inflorescences, **C** Leaves, **D** Young inflorescence, **E** Infructescence, **F** Mature cupule.

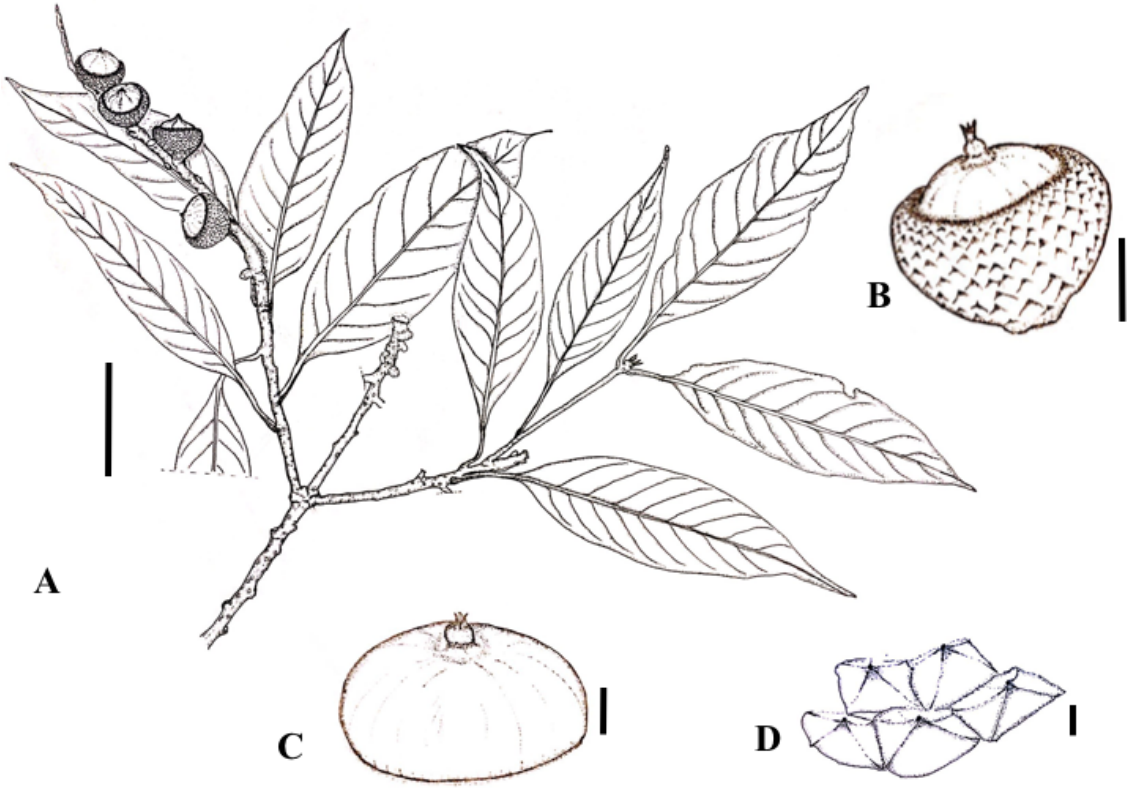

FIGURE 2.6. Illustrated of *Lithocarpus hongiaoensis* Ngoc & Binh: **A** Fruiting twigs, **B** Mature fruit, **C** Mature nut, **D** Cupule scales. Scale bars **A** = 5 cm, **B** = 1 cm, **C** = 0.5 cm, **D** = 2 mm. Drawn by Nguyen Van Ngoc & Hoang Thi Binh.

Chapter III

Lithocarpus vuquangensis (Fagaceae), a new species from Vu Quang National Park, Vietnam

Abstract

Lithocarpus vuquangensis Ngoc & Hung is described from Vu Quang National Park, North Central Vietnam. The morphological comparison and phylogenetic analysis based on *rbcL*, *matK* and ITS provided evidence that the new species was not assignable to any of the previously known taxa in Vietnam and its surrounding countries. The description, photographs, preliminary conservation status and DNA barcode sequences are also provided for the new species.

Key words

Fagaceae, *Lithocarpus*, new species, phylogeny, taxonomy, Vietnam, Vu Quang National Park.

Introduction

It has been known that species richness of the genus *Lithocarpus* Blume (Fagaceae Dumorier) is high in Vietnam where 120 species and two varieties have been reported including the recently published species, *L. dahuoaiensi*s Ngoc & L. V. Dung (Ban 2003, Ho 2003, Ngoc et al. 2016). Here, we describe an additional new species of *Lithocarpus* from Vu Quang National Park located in Ha Tinh Province, North Central Vietnam (Fig. 3.1).

Vu Quang National Park covers an area of ca. 56,000 ha from lowlands (alt. 10–300 m) to the highlands (the highest peak of Rao Co, alt. 2,286 m). Two new species of mammals (Sao La - *Pseudoryx nghetinhensis*, Artiodactyla; and the world's largest

muntjac -*Muntiacus vuquangensis,* Cetartiodactyla) were discovered from this national park in the 1990s (Dung et al. 1993, 1994). The vegetation is diverse along the elevation gradient and five major forest types are recognized: lowland forests (alt. 10–300 m), hill forest (alt. 300–1,000 m), medium montane forest (alt. 1,000–1,400 m), montane forest (alt. 1,400–1,900 m) and upper montane forest (alt. 1,900–2,100 m) (Kuznetsov 2001, Vu Quang National Park Management Board 2014). Until now, 1,678 species of vascular plants including many endemic and rare species have been reported (Vu Quang National Park Management Board 2014, Tagane et al. 2016). As for Fagaceae, one species of *Castanea* Mill, nine species of *Castanopsis* (D. Don) Spach., 12 species of *Quercus* L., and 37 species of *Lithocarpus* Blume have been recorded from the National Park, among which ten species have been listed in Viet Nam Red Data Book (Ban et al. 2007, Hung et al. 2014). In addition, natural populations of *Trigonobalanus verticillata* Forman were discovered during our recent botanical surveys in the National Park in 2016 (voucher specimens: *Yahara et al. V5764* & *V5766,* DLU, FU, the herbarium of Vu Quang National Park), which brings the number of Fagaceae genera in the region up to five.

From 2015 to 2016, floristic expeditions were carried out in Vu Quang National Park, and trees of the genus *Lithocarpus* were discovered that did not match any described species. Here, we describe and name it as *Lithocarpus vuquangensis* Ngoc & Hung, sp. nov. accompanying with its photographs and the morphological comparison with related species. In addition to the morphological examination, DNA sequences and phylogenetic analysis are extremely helpful for identifying and delimiting species (Hebert and Gregory 2005, Dick and Webb 2012). Here, we sequenced parts of the DNA barcode regions *rbcL, matK* (CBOL Plant Working Group 2009) and ITS (China Plant BOL Working Group 2011), and examined the phylogenic relationship of *L. vuquangensis* and its related taxa.

Materials and methods

Morphological observations

The morphological traits of the new species were compared with its putative relatives based on systematic literature (Camus 1948, Huang et al. 1999, Ban 2003, Ho 2003, Phengklai 2008) and also examined more than three hundred dried specimens kept in the following herbaria: BKF, DLU, FOF, HN, KYO, P, RUPP, TI and VNM as well as digitized plant specimen images available on the web of JSTOR Global Plants (https://plants.jstor.org/), Muséum National d'Histoire Naturelle (https://science.mnhn.fr/) and Chinese Virtual Herbarium (http://www.cvh.org.cn/).

DNA extraction and sequencing

Total DNA was extracted from 17 silica-gel dried leaf pieces collected in the field. DNA extraction was performed using the CTAB method (Doyle & Doyle 1987) with minor modifications described in Toyama *et al*. (2015). Two chloroplast DNA barcode regions, *rbcL* and *matK*, were amplified and sequenced following published protocols (Kress et al. 2009, Dunning and Savolainen 2010). In addition, the internal transcribed spacer (ITS) region was sequenced using the protocol of Rohwer et al. (2009) with minor modification in PCR amplification using the Tks GflexTM DNA Polymerase (Takara Bio Inc., Japan).

Phylogenetic analysis

A total of 16 accessions representing 15 species of *Lithocarpus*, collected from throughout Vietnam, were analyzed (Table 3.1). In addition, *Trigonobalanus verticillata* was used as an outgroup in the phylogenetic analysis. The sequence alignment was performed by ClustalW with default parameters implemented in MEGA v 7.0.25 (Kumar et al. 2016), and subsequently adjusted manually.

Bayesian inference (BI) of phylogeny was performed on the concatenated data set of three genes (*rbcL*, *matK* and ITS) using MrBayes v. 3.2 (Huelsenbeck and Ronquist 2001, Ronquist et al. 2012). The hierarchical likelihood ratio test (hLRTs) and Akaike Information Criterion (AIC) were used to select the best model of evolution using MrModeltest v. 2.3 (Nylander 2004). The nucleotide substitution model was set to GTR+γ as selected by MrModeltest. Four independent Markov Chain Monte Carlo (MCMC) runs of four chains each were run for 10,000,000 generations sampling every 1,000 generations. The programs Tracer v. 1.6 (Rambaut et al. 2014) was used to examine marginal prior and posterior densities of MCMC outputs. Each run produced 10,001 trees, and we used a relative burnin of 25 % for diagnostics. Consequently, 7,501 trees of each run were sampled to generate the summary tree and posterior probabilities distributions. The summary tree was visualized and edited with FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

The morphological comparison showed that *Lithocarpus vuquangensis* is most similar to *L. nantoensis* (Hayata) Hayata distributed in Taiwan, in having entire leaf margin, mostly solitary, rarely 2 or 3 clustered cupules, cupules not completely enclosing nut, and glabrous nut. The Vietnamese species sharing the above diagnostic feature of *L. vuquangensis* are *L. hongiaoensis, in ined.* (Ngoc et al. in review), and *L. vinhensis* A. Camus. However, the new species is clearly different from all the three in the following points: *L. vuquangensis* is distinguished from *L. nantoensis* by its fewer secondary veins (7–10 pairs vs. 10–15 pairs), shorter infructescences (4–7 cm long vs. 16 cm long), longer fruiting stalks (4–6 mm long vs. almost sessile), bigger nut size (1.7–2.0 cm high by 2.1–2.4 cm in diam. vs. 1.4–1.7 cm high by 1.5–1.6 cm in diam.) and larger basal scar of the nut (ca. 1.1 cm in diam. vs. 0.5–0.8 cm in diam.). *Lithocarpus vuquangensis* is distinct

from *L. hongiaoensis* by its shorter petioles (1–1.5 cm long vs. 2.1–3 cm long), shorter infructescences (4–7 cm long vs. 10 cm long), longer fruiting stalks (4–6 mm long vs. almost sessile), arrangement of scales on the cupule (scales arranged into concentric rings vs. imbricate, not forming rings), and larger nut size (1.7–2.0 cm long, 2.1–2.4 cm in diam. vs. 0.6–0.8 cm long, 1.2–1.5 cm in diam.). The new species differs from *L. vinhensis* in having fewer secondary veins (7–10 pairs vs. 11–12 pairs), shorter infructescences (4–7 cm long vs. 10 cm long), and larger nut size (1.7–2.0 cm long, 2.1–2.4 cm in diam. vs. 0.9–1 cm long, 1 cm in diam.). More detail comparison among these four species were shown in Table 3.2.

In the molecular phylogenetic tree (Fig. 3.2), *L. vuquangensis* is sister to *L. hongiaoensis* with the posterior probability of 0.94. One nucleotide substitution in *rbcL*, six in *matK* and six in ITS were found between these two species. On the other hand, *L. vinhensis*, another Vietnamese species most similar to *L. vuquangensis*, is placed in a separated clade which includes *L. longipedicellatus, L. ombrophilus, L. gigantophyllus, L. licentii, L. pseudomagneinii* and *L. lemeeanus*, with a posterior probability 0.93.

Both *Lithocarpus vuquangensis* and *L. vinhensis* were collected in Vu Quang National Park, but these two species occur in different altitudes: *L. vuquangensis* was found between 1,500 m and 1,700 m altitude, while *L. vinhensis* was found at the lower elevation, below 1,100 m.

Discussion

Phylogenetically, *L. vuquangensis* is sister to *L. hongiaoensis in ined.* collected from Lam Dong Province located in southern Vietnam. These two species are morphologically distinguished in their length of infructescences and fruiting stalk, the arrangement of cupule bracts, nut size, and other characteristics as summarized in Table

3.2. The further molecular phylogenetic studies using additional DNA markers are needed to clarify the relationship between *L. vuquangensis* and *L. hongiaoensis*. However, morphological differences are distinct enough to distinguish them as different species.

Lithocarpus vuquangensis is also morphologically similar to *L. vinhensis* in having entire leaf margin, solitary cupules not completely enclosing nut, scales arranged into concentric rings and glabrous nut, but these two species are not closely located in our phylogeny. This morphologically similarity may have evolved in the similar habitat of the montane evergreen forest in Vu Quang National Park. Whereas *L. vuquangensis* and *L. vinhensis* were collected at 1,518 m and 1,062 m, respectively, altitudinal distributions of the two species may be overlapping in the montane evergreen forest.

The morphological comparison provided evidence to distinguish *L. vuquangensis* from a Taiwanese species, *L. nantoensis*, although the relationship between them remain to be clarified by further molecular phylogenetic studies.

Taxonomy

Lithocarpus vuquangensis Ngoc & Hung, **sp. nov.**

Fig. 3.3

Diagnosis. Similar to *Lithocarpus nantoensis*, *L. hongiaoensis* and *L. vinhensis*, but distinguished from *L. nantoensis* mainly by its fewer secondary veins, shorter infructescences, longer fruiting stalk, bigger nut size, and larger scar size of the nut, from *L. hongiaoensis* by its much shorter petioles and infructescences, longer fruiting stalk, scales united into concentric rings, and much bigger nut size and *L. vinhensis* by having fewer secondary veins, shorter infructescences and much larger nut size (Table 3.2).

Type. VIETNAM. Ha Tinh Province, Vu Quang National Park, in lower montane forest, along trail to the summit of Mt. Rào Cổ, alt. 1518 m, 18°12'12.2"N, 105°23'15.3"E, 22 June 2016, *Yahara T., Nguyen Van Ngoc, Toyama H., Tagane S., Okabe N., Nguyen Viet Hung V5743* (holotype: KYO!; isotypes: DLU!, FU!, HN!, K!, P!, VNM!).

Description. Trees, ca. 15–20 m tall; young branches mostly glabrous, yellowish *in vivo*, reddish brown *in sicco*. Leaves alternate, spirally arranged, blade narrowly elliptic to lanceolate, 7.5–11 × 2.3–3.6 cm, crunchy, glabrous adaxially, white farinose abaxially, apex long acuminate, acumen up to 1.2 cm long, base cuneate to attenuate, margin entire and wavy; midrib flat or slightly prominent near base adaxially, prominent abaxially, greenish yellow *in vivo*, reddish brown *in sicco,* secondary veins 7–10 pairs, at an angle of 40–50 degree from the midrib, prominent abaxially, tertiary veins scalariform, faintly visible to invisible on both sides; petiole ca. 1–1.5 cm long, glabrous, terete. Male inflorescence a spike, 7–8.5 cm long. Male flower solitary; calyx 6-lobed, lobes ovate, 0.5–0.6 mm × 0.4–0.5 mm, pubescent on both surfaces; stamens 12, 0.7–0.9 mm long, anthers 0.1–0.15 mm long. Infructescences erect, woody spike, up to 7 cm long, axis ca. 2 mm thick at base, grayish brown, lenticellate. Cupule solitary, broadly obconical to saucer-shaped, 1.4 cm long. 1.8 cm in diam., enclosing only basal to 1/4 of the nuts; scales triangular, arranged into 4–5 concentric rings, apex shortly acuminate, densely covered with tawny minute hairs; fruiting stalk ca. 4–6 mm long, 4–7 mm in diam.; Nut obovoid or globose, 1.7–2.0 cm long, 2.1–2.4 cm in diam., glabrous, dehiscent; basal scar concave, ca. 1.1 cm in diam.

Phenology. Mature fruits were collected in June.

Distribution. Vietnam (so far known only from Vu Quang National Park, Ha Tinh Province) (Figure 3.1).

Etymology. The specific epithet is derived from its type locality, Vu Quang National Park.

GenBank accession No. *Yahara et al. V5743*: LC319671 (*rbcL*), LC319670 (*matK*), KY786083 (ITS).

Preliminary conservation status. Critically Endangered (CR). In our field observation, we found less than 10 individuals along the trail to the summit of Mt. Rào Cổ, in lower montane forest. The habitat is inside the protected areas of Vu Quang National Park, but based on criterion D of the IUCN Red List criteria (IUCN 2012), this species is qualified as CR. Further intensive inventories are needed to find additional populations in Vu Quang National Park and its surrounding areas.

Acknowledgements

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Legends

TABLE 3.1. List of taxa used in this study with vouchers and GenBank accession number.

Species	Vouchers	GenBank accession number		
		rbcL	***matK***	**ITS**
Lithocarpus aggregatus	*Tagane et al. V6288* (DLU, FU)	LC318967	LC318550	MF770309
Lithocarpus bidoupensis	*Tagane et al. V4320* (DLU, FU, VNM)	LC318961	LC318547	KY940070
Lithocarpus coalitus	*Tagane et al. V4191* (DLU, FU, VNM)	LC318959	LC318545	MF770305
Lithocarpus dahuoaiensis	*Ngoc et al. V3194* (DLU, FU, HN, K, KYO, P, VNM)	LC318953	LC318551	KY436002
	Ngoc et al. V5404 (DLU, FU)	LC318964	LC318548	MF770307
Lithocarpus gigantophyllus	*Ngoc et al. V3185* (DLU, FU)	LC318951	LC318538	MF770299
Lithocarpus hancei	*Ngoc et al. V5111* (DLU, FU)	LC318963	LC318970	MF952868
Lithocarpus hongiaoensis	*Ngoc et al. V3235* (DLU, FU)	LC318956	LC318542	KY851759
Lithocarpus lemeeanus	*Tagane et al. V4273* (DLU, FU)	LC318960	LC318546	MF770306
Lithocarpus licentii	*Ngoc et al. V3205* (DLU, FU)	LC318954	LC318540	MF770301
Lithocarpus longipedicellatus	*Nguyen et al. V3813* (DLU, FU)	LC318958	LC318544	MF770304
Lithocarpus ombrophilus	*Yahara et al. V3000* (DLU, FU)	LC318949	LC318420	MF770297
Lithocarpus pseudomagneinii	*Ngoc et al. V3223* (DLU, FU)	LC318955	LC318541	MF770302
Lithocarpus stenopus	*Ngoc et al. V3187* (DLU, FU)	LC318952	LC318539	MF770300
Lithocarpus vinhensis	*Nguyen et al. V3787* (DLU, FU)	LC318957	LC318543	MF770303
Lithocarpus vuquangensis	*Yahara et al. V5743* (DLU, FU)	LC319671	LC319670	KY786083
Trigonobalanus verticillata	*Yahara et al. V5764* (DLU, FU)	LC318965	LC318549	MF770308

Table 3.2. Morphological comparison of *Lithocarpus vuquangensis* with three related species: The measurements of *L. nantoensis* is derived from Hayata (1911), Liao (1996), Huang et al. (1999) and from digitized type specimen image (*Kawakami & Mori 1157*, TI); The measurements of *L. vinhensis* and *L. hongiaoensis* are derived from Camus (1948) and Ngoc et al. (in review), respectively.

Characters	***L. vuquangensis***	***L. nantoensis***	***L. hongiaoensis***	***L. vinhensis***
Leaf margin	Entire	Entire	Entire	Entire
Leaf surface	Glabrous adaxially, adaxially white farinose	Abaxially glaucous to light green and with adherent, waxy scalelike trichomes	Glabrous upper, adherent waxy scale abaxially	Glabrous adaxially, covered with very short white villi abaxially
Leaf blade size	7.5–11 × 2.3–3.6 cm	12–16 × 2.5–3.5 cm	9.6–14.5 × 2.5–3.8 cm	7.5 cm × 3 cm
Petiole length	1–1.5 cm long	0.7–1.3 cm long	2.1–3 cm long	1 cm long
Number of secondary veins	7–10 pairs	10–15 pairs	8–11 pairs	11–12 pairs
Infructescences length	4–7 cm long	16 cm long	12.5–16.5 cm long	10 cm long
Fruiting stalk length	Ca. 4–6 mm long, 4–7 mm in diam.	Almost sessile	Sessile to ca. 2 mm long	5–6 mm long
Cupule	Solitary, 0.6–0.9 cm high by 1.8–2.2 cm in diam.	Solitary, 1.2–1.5 cm in diam.	Solitary, 1–1.2 cm high by 1.8–2.1 cm in diam.	Solitary, 1.2–1.3 cm high by 0.8–1 cm in diam.
Scale arrangement	Arranged into concentric rings	Arranged into concentric rings	Imbricate	Arranged into concentric rings
Nut size	1.7–2.0 cm high by 2.1–2.4 cm in diam.	1.4–1.7 cm high by 1.5–1.6 cm in diam.	0.6–0.8 cm high by 1.2–1.5 cm in diam.	0.9–1 cm high by 1 cm in diam.
Nut enclosure by cupule	Only basal to 1/4 of the nut	Only basal part of the nut	Enclosing ca. 1/3–1/2 of the nut	Enclosing ca. 1/3–1/2 of the nut
Basal scar of the nut	Concave, ca. 1.1 cm in diam.	Concave, 0.5–0.8 cm in diam.,	Slightly concave, 1.2–1.4 cm in diam.	Nearly flat

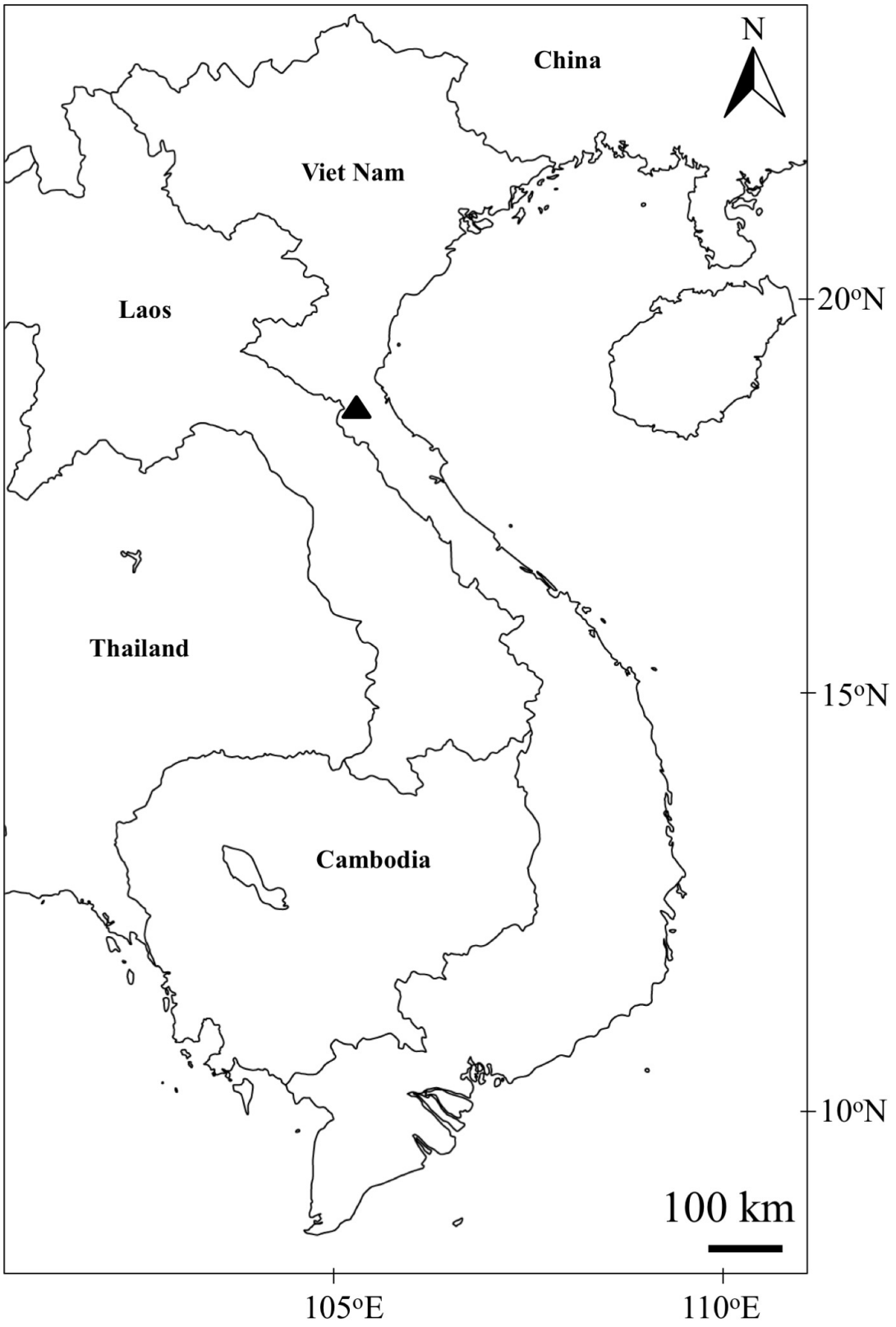

Figure 3.1. Location of Vu Quang National Park (Black triangle), type locality of *Lithocarpus vuquangensis*.

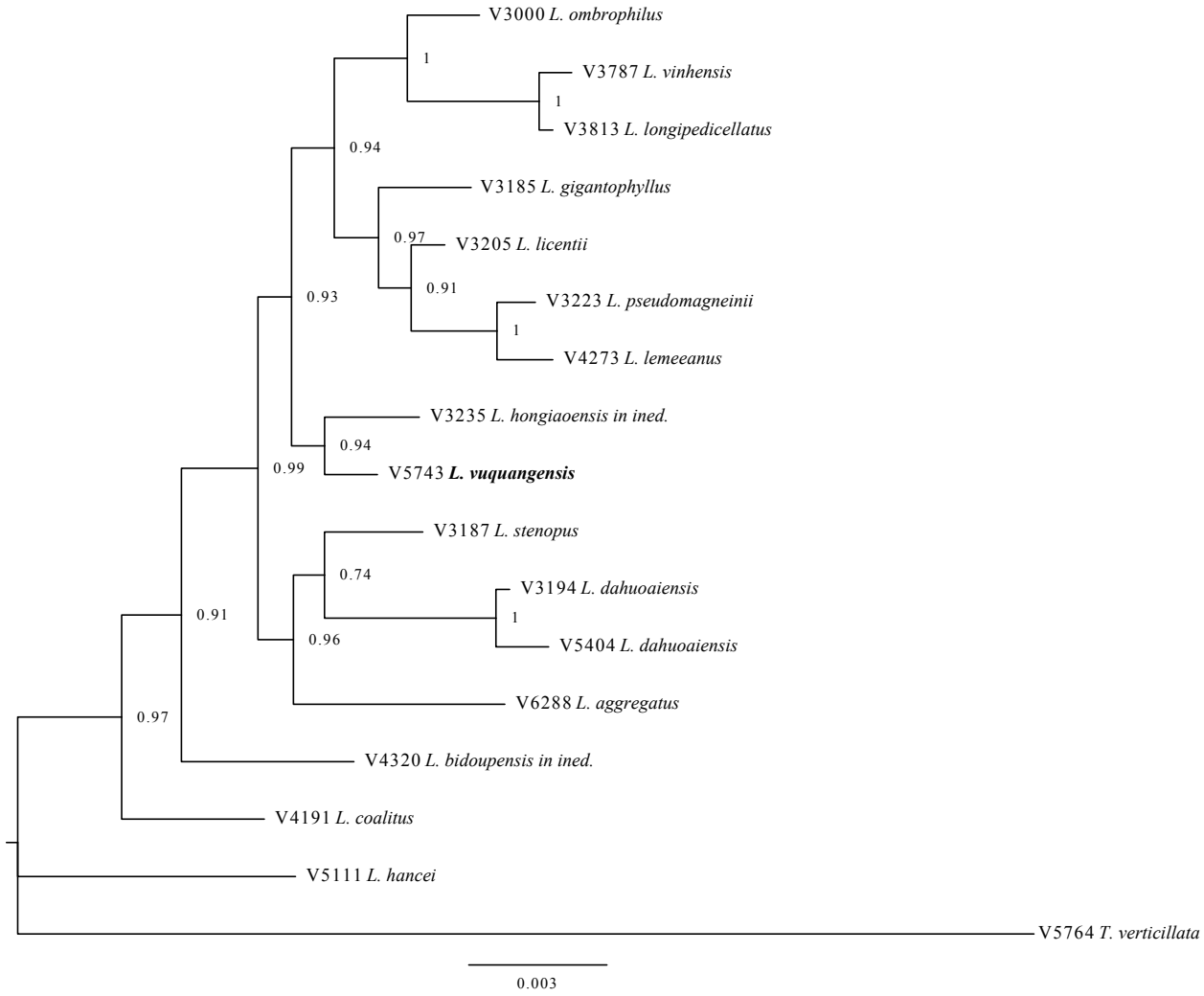

Figure 3.2. Bayesian phylogeny estimate of 15 taxa of *Lithocarpus* and one *Trigonobalanus verticillata* (as an outgroup) based on combined *rbcL*, *matK,* and ITS sequences. Branches are labeled with posterior probabilities greater than 0.7.

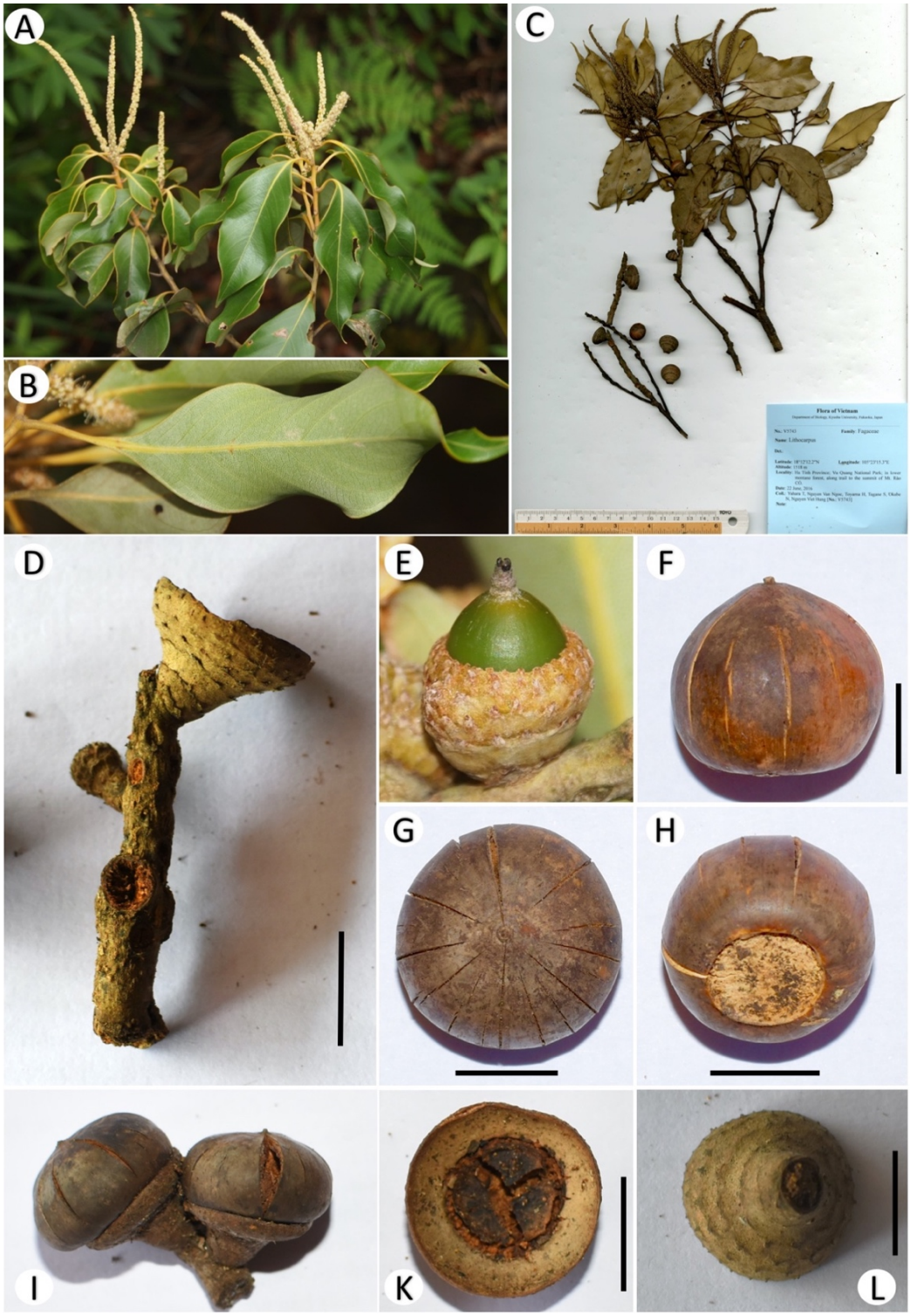

Figure 3.3: *Lithocarpus vuquangensis* Ngoc & Hung: (**A**) Branch with male inflorescences, (**B**) Lower leaf surface, (**C**) Holotype (KYO), (**D**) Spike with cupule, (**E**) Young acorn, (**F–H**) Side view, top view and scar of the mature nut, respectively, (**I**) A part of infructescence, (**K & L**) Inside and outside of the cupule. (**C**, **D, F–L**) From *Yahara et al. V5743*. Scale bars (**D**) = 2 cm, (**F–H, K, L**) = 1 cm.

Chapter IV

A taxonomic study of *Lithocarpus vestitus* complex (Fagaceae) based on next-generation sequencing and morphological observation.

Abstract

We revised the taxonomy of *Lithocarpus vestitus* and close relatives found in Vietnam, Laos, Cambodia and Thailand with field observations, morphological comparisons of fertile and also sterile materials, and molecular analyses based on multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq). Based on evidence from morphological observations and the MIG-seq-based phylogenetic tree, we recognized 13 species in the *vestitus*-complex from the four countries, three of which are newly described in this paper: *L. chinhii* sp. nov., *L. pierreioides* sp. nov. and *L. pseudoannamensis* sp. nov. Four species of the complex were lectotypified.

Key words

Fagaceae, *Lithocarpus*, MIG-seq, Next Generation Sequencing, new species, taxonomy.

Introduction

Lithocarpus Blume (1826), comprising more than 300 species (Camus 1934–1954, The Plant list 2013), is the second largest genus in the family Fagaceae. In classic taxonomic studies, the genus was divided into two or three smaller genera: *Lithocarpus* s.str., *Cyclobalanus* (End.) Orest. and *Pasania* (Miq.) Orest. by Orested (1867, 1871) and Schwarz (1936), or *Pasania* and *Synaedrys* Lind. by Prantl (1887, 1894) and Koidzumi (1916). Later, Rehder & Wilson (1916) adopted a broader concept of *Lithocarpus* and this concept has been accepted by many taxonomists including Rehder (1919, 1929), Barnett (1942, 1944), Camus (1954) and Forman (1966).

While *Lithocarpus* is widespread from Japan and China to New Guinea, the center of its diversity can be found in continental Asia itself, mainly from China (123 spp.; Huang et al. 1999) to continental Southeast Asia (169 spp. for Indo-China; Camus 1948) including Vietnam (118 spp.; Ho 2003, Ban 2003, Ngoc et al. 2016) and Thailand (59 spp.; Barnett 1940, Phengklai 2008, Strijk et al. 2014a, 2014b). One North American species (*Lithocarpus densiflorus* (Hook. & Arn.)

Rehder) is now treated as a separate monotypic genus (*Notholithocarpus*) based on molecular data (Manos et al. 2008).

In Indo-China, there is a species complex having the following traits that are commonly observed in *Lithocarpus*: entire simple leaves not densely hairy when mature and more or less narrow (more than 2.5 times longer than wide), infructescences 10–20 cm long, cupule with short scales on the wall, sessile or subsessile, in clusters of 3, not or slightly connate when mature, enclosing approximately half or less of the nut, and nut usually concave at base, more or less broader than tall. Because these species lack easily recognizable traits such as densely hairiness, wider leaves, very long infructescence, distinct pedicel of cupule, elongated scales on cupule wall, deeply connate cupule, the nut mostly covered by cupule and acorns longer than wide and distinctly convex at base, the taxonomy of those species remain very confusing. According to the key and description of *Pasania* in Flore du Indo-Chine (Hickel & Camus 1921) the following species are included in this group: *Lithocarpus annamensis* (Hickel & A.Camus) Barnett*, L. bacgiangensis* (Hickel & A.Camus) A.Camus*, L. dinhensis* (Hickel & A.Camus) A.Camus, and *L. vestitus* (Hickel & A.Camus) A.Camus. Here we designate this group as *Lithocarpus vestitus* complex. Among them, *L. bacgiangensis* and *L. vestitus* are described from Vietnam, *L. annamensis* from Laos and Vietnam, and *L. dinhensis* from Cambodia and Vietnam. In addition, after the floristic treatment of Hickel and Camus (1921), the following four species and two subspecies of the complex were described from Vietnam: *L. pierrei* (Hickel & A.Camus) A.Camus (1931), *L. microspermus* A.Camus (1935), *L. pseudovestitus* A.Camus (1939), *L. microspermus* subsp. *mekongensis* A.Camus (1948), *L. bacgiangensis* subsp. *bentramensis* A.Camus (1948), and *L. ailaoensis* A.Camus (1948). However, Phengklai (2008) reduced *L. microspermus* to a synonym of *L. vestitus*, and Dr. Tem Smitinand annotated the type specimens of *L. annamensis*, *L. bacgiangensis,* and *L. bacgiangensis* subsp. *bentramensis* as *L. thomsonii* and those of *L. dinhensis* and *L. microspermus* are as *L. vestitus*. More recently, Ho (2003) treated *L. annamensis, L. bacgiangensis, L. bentramensis, L. dinhensis* and *L. vestitus* as distinct species, indicating that the taxonomy of the *Lithocarpus vestitus* complex is still controversial.

The purpose of this paper is to resolve the taxonomy of the *Lithocarpus vestitus* complex based on evidence obtained from our field observations, morphological studies of specimens in herbaria, and molecular analyses. In our molecular approach, we employ genome-wide sequences using next-generation sequencing (MIG-seq; Suyama & Matsuki 2015) to reconstruct

phylogenetic relationships among species in the *L. vestitus* complex. MIG-seq provides SNP markers of relatively short sequence reads spread throughout the genome, as opposed to long continuous stretches of genomic DNA. MIG-seq is similar to RAD-seq (Cavender-Bares et al. 2015, Fitz-Gibbon et al. 2017), but MIG-seq markers are obtained with a PCR-based procedure without restriction enzyme digestion steps and more widely applicable to samples with low-quality DNA and/or small quantities of DNA (Suyama and Matsuki 2015).

The evidence obtained from all our examinations supports the recognition of *L. bacgiangensis, L. braianensis, L. dinhensis, L. mekongensis, L. microspermus, L. pierrei*, *L. pseudovestitus*, *L. vestitus* and three new species from Cambodia and Vietnam as distinct species. In addition, four species of the complex were lectotypified in this study.

Materials and methods

Taxon sampling

We made field collections as a part of the plant diversity assessment project using standardized rectangular plots of 100 m × 5 m and general sampling with a specific attention to Fagaceae at various locations of Indo-China and Thailand (Yahara et al. 2012, Zhang et al. 2016). We collected 45 specimens of *Lithocarpus* from Cambodia, 24 from Laos and 54 from Thailand. In Vietnam, we carried out field surveys more intensively, from Feb. 2014 to Feb. 2017, collecting 315 specimens in five National Parks (NP) and two Nature Reserves (NR), including Hoang Lien NP, Ba Vi NP, Vu Quang NP, Bach Ma NP, Bidoup-Nui Ba NP, Ngoc Linh NR, and Hon Ba NR. Here, we established 26 plots along an altitudinal gradient from 58 m to 2933 m. In addition, we made general collections of Fagaceae without placing plots in Pu Mat NP, Ba Na NR, Son Tra Peninsula, Dong Nai NR and the surrounding areas of Bidoup-Nui Ba NP (Fig. 4.1). For each specimen, we also collected leaf fragments and dried them with silica-gel for DNA extraction.

For the *L. vestitus* complex, we collected *L. vestitus* and *L. microspermus* from their type localities, and *L. dinhensis*, *L. pierrei*, *L. pseudovestitus*, *L. bacgiangensis*, *L. mekongensis* from non-type localities (Table 4.1). *Lithocarpus braianensis* is morphologically distinct from the *L. vestitus* complex by its solitary fruits but included in this study because our preliminary phylogenetic study suggested its affinity with the *L. vestitus* complex. The specimens *Nguyen et al. V3196*, *Nguyen et al. V3260*, *Nguyen et al. V3261* from Lam Dong Province, Vietnam and

Tagane et al. 5514 collected from from Bokor National Park, Cambodia are also included in this study because those specimens have morphological features of the *L. vestitus* complex.

Three other species of the *L. vestitus* complex, *L. ailaoensis, L. annamensis* and *L. bentramensis,* were not collected in the field surveys, but J.S. Strijk examined the specimens at Muséum national d'Histoire naturelle (P) and selected their lectotypes.

DNA extraction

Total genomic DNA was isolated from silica-gel dried leaf fragments by the CTAB method (Doyle and Doyle 1987) with minor modifications described in Toyama et al. (2015). Partial sequences of the large subunit ribulose-1,5-bisphosphate carboxylase oxygenase (*rbcL*), maturase K (*matK*) and internal transcribed spacer region of ribosomal DNA (ITS) were amplified with the given primer sets (Table 4.2) and Tks GflexTM DNA Polymerase (Takara Bio Inc., Kusatsu, Japan) according to published protocols of Kress et al. (2009), Dunning & Savolainen (2010) and Rohwer et al. (2009), respectively.

New genotyping method with next generation sequencer (MIG-seq)

The DNA extracted from 322 specimens of *Lithocarpus* spp. were diluted to *ca*. 10 ng/µl and used as templates to amplify thousands of short sequences (loci) from a wide variety of genomes with a standard PCR protocols according Suyama and Matsuki (2015). The presence/absence genotype information of each locus in each individual was used for phylogenetic tree reconstruction. MIG-seq library was constructed as described in Suyama and Matsuki (2015). As the 1st PCR, multiple non-repetitive regions from various inter-simple-sequence repeats (ISSRs) were amplified from genomic DNA by multiplexed PCR with tailed ISSR primers. The products of the first PCR were diluted 50 times with deionized water, and were subsequently used as the templates for the 2nd PCR (tailed PCR). This step enables the addition of complementary sequences for the binding sites of Illumina sequencing flow cell and index (barcode) for each sample to the 1st PCR products, using dual indexed forward and reverse primers. Then, 3 µl of each 2nd PCR product was pooled in a single mixture library. The mixture was then purified and the size range of 350–800 bp were isolated by a Pippin Prep DNA size selection system (Sage Science, Beverly, MA, USA). The final concentration was measured by quantitative PCR 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 inference based on next generation DNA sequencing – MIG-seq

The raw data from 322 samples was pretreated and completed quality control using Fastx-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and TagDust program (Lassmann et al. 2009) following Suyama and Matsuki (2015). Then, the quality-filtered reads were used as input data for ustacks, cstacks and sstacks in Stacks software package version 1.35 (Catchen et al. 2011) for SNP detection. We obtained a table of presence/absence of loci in each individual after the population pipeline with following settings: all samples belong to the same population, and threshold frequency of haplotype count in a population (r) = 0.75, a threshold one-order higher than 0.01. The presence/absence (1/0) data of loci were used to compute distance matrix, construct a neighbor-joining (NJ) tree, and examine the reliability of tree topology by bootstrapping with 1000 replicate using PHYLYP ver. 3.695 (Felsenstein 2005) as follows; First, we used Seqboot for 1000 times resampling from presence/absence data of loci. Second, distance matrices were computed with Restdist program. Third, the distance matrices were used to infer the neighbor-joining (NJ) trees. Finally, a consensus tree was constructed with Consense. The final tree was visualized with FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Before focusing on the *L. vestitus* complex, we made a phylogenetic analysis using all 322 samples and then confined the phylogenetic analysis to 23 samples of the *L. vestitus* complex. *Trigonobalanus verticillata* Forman (*Yahara et al. V5764*) was used as an outgroup.

Morphological and taxonomic comparison

Base on the results of the phylogenetic analysis, we carefully reexamined morphological traits of each species of the *L. vestitus* complex as well as its related species. Then the taxonomy of the complex was revised through taxonomic literature review and thorough examination of specimens kept in the herbaria ANDA, BKF, DLU, HN, KYO, P, and VNM as well as digitized plant specimen images available on the web of JSTOR Global Plants (https://plants.jstor.org/) and Chinese Virtual Herbarium (http://www.cvh.org.cn/). If two OTUs are morphologically distinguishable and also not monophyletic in the phylogenetic trees, we regarded them as two distinct species.

Results

Phylogenetic analysis using MIG-seq

A total of 18,226 loci were detected and used for the phylogenetic analysis. In the neighbor-joining (NJ) tree based on MIG-seq of 23 *Lithocarpus* samples and one sample of *Trigonobalanus* (Fig. 4.2), two or more samples are clustered as *L. dinhensis, L. pseudovestitus, L. microspermus, L. bacgianensis, L. pseudoannamensis, L. mekongensis* and *L. braianensis*. In all of those species, samples of the same species clustered together and a bootstrap value for the monophyly of each species exceeded 0.94 except for 0.74 in *L. bacgianensis*. Among those species, *L. mekongensis* and *L. braianeisis* were sister to each other but the monophyly of those two was supported by a bootstrap value as low as 0.54. *Lithocarpus dinhensis* and *L. pseudovestitus* were sister to each other and this clade was sister to *L. microspermus*. However, bootstrap values for those relationships were 0.50 and 0.48, respectively. Among the other four species in which only one sample was examined, *L. pierrei* and *L. chinhii* sp. nov. (see description) clustered with *L. pseudoannamensis* sp. nov. (see description) with a bootstrap value 0.86. *Lithocarpus pierreioides* sp. nov. (see description) and *L. vestitus* did not cluster with any other species with bootstrap values higher than 0.50.

Morphological observation

We made morphological observations on *L. dinhensis*, *L. pseudovestitus, L. microspermus, L. bacgianensis, L. vestitus, L. mekongensis,* and *L. braianensis* in which identities as different species were supported by the MIG-seq tree. Consequently, we found the following diagnostic traits of each species. *Lithocarpus dinhensis* is characterized by having leaves densely grayish tomentose beneath, acorn taller than wide and smaller than 1 cm, cupule covering its 1/3–1/2; *L. pseudovestitus* by leaves shorter than 10 cm with 7–8 pairs of secondary veins, cupules covering only basal part of the nut; *L. microspermus* by its leaves having many secondary veins (18–20 pairs), acorn wider than tall, cupules covering its 1/3–1/2; *L. bacgiangensis* by having leaves glabrous on both sides, acorn taller than wide, cupules covering its 1/3–1/2, nut depressed globose; *L. vestitus* by leaves oblong or elliptic-oblong, not less than 10 by 3.5 cm, 14–20 secondary veins, acorn taller than wide, cupules covering only basal of the nut, nut flat at the apex*; L. mekongensis* by having acorns more or less wider than tall, densely arranged on a short infructescences, cupules

covering only basal part of the nut, and nut depressed globose and its apex flat or concave; *L. braianensis* by its solitary acorns.

Based on the phylogenetic analyses with MIG-seq and examinations of the digital images of type specimens available on the web, the specimens kept in the herbaria in Vietnam as well as other countries, we recognize and describe three new species: *Lithocarpus chinhii* Ngoc & Binh for *Nguyen et al. V3196*, *L. pseudoannamensis* Ngoc & Binh for *Nguyen et al. V3260* and *Nguyen et al. V3261*, and *L. pierreioides* Ngoc, Tagane & Yahara for *Tagane et al. 5514*. All of *L. pseudoannamensis, L. chinhii* and *L. pierrei* that clustered with a bootstrap value of 0.86 were collected in Lam Dong Province. *Lithocarpus pseudoannamensis* was distinguished from the other two in larger leaf blade (15–23.5 × 4.5–8.5 cm *L. pseudoannamensis* vs. 12–15 × 3.5–5 cm in *L. chinhii*, 25–30 × 6–8 cm in *L. pierrei*), bigger cupules (0.5–0.8 cm high × 1.7–2.2 cm in diam. vs. 0.5–0.7 cm high × 1.2–1.4 cm in diam. in *L. chinhii,* 0.5–0.7 cm high × 1.5–1.7 cm in diam. in *L. pierrei*), bigger nuts (1.3–1.6 cm high × 1.6–1.8 cm in diam. vs. 1.4–1.6 cm high × 1.1–1.3 cm in diam. in *L. chinhii,* 1.2 cm high × 1.3 cm in diam. in *L. pierrei*), and bigger basal scar of the nut (1–1.2 cm in diam. vs. ca. 0.7–0.8 cm in diam. in *L. chinhii,* ca. 0.6–0.7 cm in diam. in *L. pierrei*). *Lithocarpus pierrei* was distinguished from *L. chinhii* by larger leaf blade (25–30 × 6–8 cm in *L. pierrei* vs. 12–15 × 3.5–5 cm in *L. chinhii*), longer petioles (1.5 cm long vs. 0.8–1 cm long), more secondary veins (15–16 pairs vs. 12–13 pairs), and nut shape (subglobose vs. obovoid or turbinate).

Lithocarpus pierreioides is morphologically similar to *L. pierrei* in having leaves entire, glabrous on both sides, cupules solitary, rarely clustered of 2 or 3, plated-shape, hairy outside, scales on cupules wall very small triangular and not united into concentric rings but distinguished by its smaller leaves (10.5–14 × 3–4.5 cm vs. 25–30 × 6–8 cm), shorter petioles (0.8–1 cm long vs. 1.5 cm long), fewer secondary veins (12–13 pairs vs. 15–16 pairs), and smaller nuts (0.5–0.7 cm high × 0.8–1 cm in diam. vs. 1.2 cm high × 1.3 cm in diam.).

We also examined the type specimens of *L. ailaoensis, L. annamensis* and *L. bentramensis* that were not collected in the field work of this study. *Lithocarpus ailaoensis* is easily recognized by its smallest leaves among the *vestitus* complex (less than 10 cm including petiole). *Lithocarpus annamensis* is most similar to *L. pseudoannamensis* but differs in its smaller leave, fewer secondary veins, shorter infructescences, smaller fruits, cupule covering only base of nut and smaller basal scar (Table 4.3). *Lithocarpus bentramensis* is morphologically similar to *L.*

bacgiangensis but distinct in its sparse fruits on a longer infructescence and a distinct pedicel of a cupule.

Discussion

Based on the MIG-seq tree (Fig. 4.2) and morphological observations described above, a total of 10 species are recognized among our samples of the *L. vestitus* complex. First, *L. microspermus* was treated as a synonym of *L. vestitus* in the Flora of Thailand (Phengklai 2008) but it is not sister to the latter and the morphological comparison provided reliable evidence that two species are distinguishable in the number of secondary veins (14–15 pairs in *L. vestitus* vs. 18–20 pairs in *L. microspermus*), the infructescence length (9–10 cm vs. 15 cm) and nut size (1.4–1.5 × 15 cm vs. 0.4–0.7 × 0.7–0.8 cm). Hickel and Camus (1921) noted in their description that *L. vestitus* (*Krempf 1913*) was collected from "Nha Trang", Laos. However, Nha Trang belongs to Khanh Hoa Province, which is located in the southern part of Vietnam. We could not examine the type specimen of *L. vestitus*, *Krempf 1913*, but based on the original description, the illustration by Camus (1948, Plate 454) and morphological observation at the field and herbarium materials (VNM), we concluded that the specimen *Poilane 4640* at VNM herbarium collected from Co Inh Pass, Nha Trang, 700 m alt., and our specimen (*Toyama et al. V1135*) collected at 617 m alt., Mt. Hon Ba, Nha Trang are identical with *L. vestitus*. As far as we examined, *L. vestitus* is endemic to Mt. Hon Ba and its vicinity, Nha Trang, Vietnam. According to the MIG-seq tree (Fig. 4.2), *L. vestitus* is not close to any other species of the *L. vestitus* complex. *Lithocarpus microspermus* was described from Lam Dong Province, where we collected the specimens V3174 and V3259 that are morphologically identical with the type specimens of *L. microspermus* (*Poilane 22355, 22259, 22413,* P!) that were annotated as *L. vestitus* by Tem Smitinand (1994). The MIG-seq tree (Fig. 4.2) and morphological observation showed that *Tagane et al. T2121* collected from Petchaburi Province, southwestern Thailand is also *L. microspermus*.

Second, *L. dinhensis,* another species that was annotated as *L. vestitus* by Tem Smitinand on the type specimen, was not sister to *L. vestitus*, and morphologically distinct from *L. vestitus* in its shorter leaves (10–14 cm × 2–5 cm vs. up to 20 cm long), fewer number of secondary veins (12–14 pairs vs. 14–15 pairs), nut enclosure 1/2 or more, smaller nut size (1 cm long × 0.8–0.9 cm in diam. vs. 1.4–1.5 cm long × 1.5 cm in diam.). In the MIG-seq tree, *L. dinhensis, L. pseudovestitus* and *L. microspermus* formed a clade but the monophyly of this clade was only

weakly supported by a low bootstrap support (0.48). On the other hand, these three species are morphologically distinguishable and the monophyly of each of the three species was supported by a bootstrap value above 0.94. Thus, we regard these three as distinct species.

Third, whereas the monophyly of *L. chinhii* (*Nguyen et al. V3196*)*, L. pseudoannamensis* (*Nguyen et al. V3260*, *V3261*) and *L. pierrei* (*Nguyen et al. V3199*) are supported by high bootstrap support of (0.86) those three are recognized as different species because those are morphologically distinct. In Lam Dong Province, we collected all of these three species, and this sympatric state without intermediates supports that those are different species.

Fourth, *L. pierreioides* (*Tagane et al. 5514*) reported as *L. pierrei* in the Picture Guide of Bokor National Park, Cambodia (Tagane et al. 2017) was not identical with genuine *L. pierrei* (Fig. 4.3). While *L. pierrei* (*Nguyen et al. V3199*) was found in the elevation 1355 m of the Lam Dong Province, *L. pierreioides* (*Tagane et al. 5514*) was collected at 530 m elevation of Mt. Bokor, Kampot Province, Cambodia. Considering the distinction in morphology (Table 4.3), separation in the MIG-seq tree and allopatric distribution, *L. pierrei* and *L. pierreioides* are treated as distinct species.

Fifth, *L. bacgianensis* is not close to any other species of the *L. vestitus* complex.

Sixth*, L. mekongensis* is sister to *L. braianensis* that has solitary nuts and were both excluded from the *L. vestitus* complex before the MIG-seq tree was available.

Among ten recognized species, lectotypification was needed for *Lithocarpus ailaoensis, L. annamensis, L. microspermus,* and *L. vestitus.* One of the authors, J. S. Strijk, examined type specimens cited in the original publications at P, and selected a specimen that best represents each species as a lectotype (see Taxonomic treatment).

Based on the conclusion that the above ten species are recognized as distinct, we could examine identities of the three other species of the *L. vestitus* complex, *L. ailaoensis, L. annamensis* and *L. bentramensis*. Consequently, we recognized all three as morphologically distinct species. Below, we provide a key to 13 species of the *Lithocarpus vestitus* complex and descriptions of three new species.

Key to the species of *Lithocarpus vestitus* complex

1. Acorn taller than wide.
   2. Cupule covering only basal of the nut.
      3. Leaves up to 20 cm long, secondary veins 14–20 pairs. ..... **1. *L. vestitus***
      3. Leaves less than 10 cm long, secondary veins 7–8 pairs. .... **2. *L. pseudovestitus***
   2. Cupule covering 1/3–1/2 of the nut.
      4. Leaves glabrous on both sides; acorn bigger than 1 cm.
         5. Leaves 14–15 secondary veins, nut depressed globose, apex depressed..... ..... **3. *L. bacgiangensis***
         5. Leaves 12–13 secondary veins; nut obovoid to turbinate, apex mucronate .... **4. *L. chinhii***
      4. Leaves densely grayish tomentose beneath, acorn smaller than 1 cm ..... **5. *L. dinhensis***
1. Acorn wider than tall.
   6. Cupule covering 1/2 or more of the nut.
      7. Leaves less than 10 cm long..... **6. *L. ailaoensis***
      7. Leaves more than 10 cm long.
         8. Leaves 25–30 cm long, secondary veins 15–16 pairs..... **7. *L. pierrei***
         8. Leaves less than 15 cm long, secondary veins 12–13 pairs..... ..... **8. *L. pierreioides***
   6. Cupule covering less than 1/2 of the acorn.
      9. Cupule covering only basal of the nut.
         10. Fruits sparse on a long infructescences; nut turbinate or conical, apex mucronate..... ..... **9. *L. bentramensis***
         10. Fruits densely on a short infructescences; nut depressed globose, apex ± flat or sometimes concave.
            11. Leaves with 12–15 secondary veins, infructescences 13–18 cm long cm..... ..... **10. *L. mekongensis***
            11. Leaves with 11–12 secondary veins, infructescences 5–10 cm long..... ..... **11. *L. annamensis***
      9. Cupule covering 1/3–1/2 of the nut.
         12. Leaves with 12–14 secondary veins; nut strongly depressed globose, apex slightly flat or sometimes concave ..... **12. *L. pseudoannamensis***
         12. Leaves with 18–20 secondary veins; nut conical, apex mucronate..... ..... **13. *L. microspermus***

Taxonomic treatment

Lithocarpus ailaoensis A.Camus, Chênes Atlas 3: 109 (1948).

TYPE: Vietnam, "Annam, Col d'Ailao, prov. de Quang Tri", 15 July 1935, *Poilane 24887* (lectotype **designated here**: P [P00744694!]; isolectotype: P [P00744695!]).

Specimens examined: Vietnam. Quang Tri Province, "Annam, Col d'Ailao, prov. de Quang Tri", 15 July 1935, *Poilane 24887* [fr.] (P!).

Distribution: Vietnam (known only from the type locality, Quang Tri Province).

Lithocarpus annamensis (Hickel & A.Camus) Barnett, Trans. & Proc. Bot. Soc. Edinburgh 34: 174 (1944).

Pasania annamensis Hickel & A.Camus Ann. Sci. Nat., Bot. 10, 3: 394 (1921); [P. H. Lecomte et al.] Fl. Indo-Chine 5: 980 (1929).

TYPE: Vietnam, "In locis arenosis prope Hué", September 1877, *F.J.Harmand s.n.* (lectotype **designated here**: P [P00744505!]; isolectotype: P [P00744504!, P00744506!]).

Specimens examined: Vietnam. Thua Thien Hue Province, "In locis arenosis prope Hué", September 1877, *F.J.Harmand* [fr.] (P!); Da Nang [Tourane], "Annam: Liên-Chiên près Tourane", 10 August 1923, *Poilane 7452* [fr.] (VNM!); Quang Binh Province, Phong Nha, 31 August 2012, *Phương 3281* [fr.] (HN!). Laos. "entre Ban. Dong et Paksong pro: Bassac plateau des Boloven 1000 à", 1100 m alt., 26 September 1928, *Poilane 15721* [fr.] (VNM!).

Distribution: Vietnam: Lang Son, Hue, Lam Dong, Dong Nai. Laos, China (Yunnan).

Lithocarpus bacgiangensis (Hickel & A.Camus) A.Camus, Rivièra Sci. 18: 39 (1931).

Pasania bacgiangensis Hickel & A.Camus Ann. Sci. Nat., Bot. 10, 3: 396 (1921); [P. H. Lecomte et al.] Fl. Indo-Chine 5: 986 (1929).

TYPE: Vietnam, "Tonkin, prov. Bac Giang : Forêt de Pho Vi", 20 December 1913, *A.J.B.Chevalier 29659* (holotype: P [P00744503!]; isotypes: [P00744511!, P00744512!, P00744513!]).

Specimens examined: Vietnam, Bac Giang Province, "Tonkin, prov. Bac Giang: Forêt de Pho Vi", 20 December 1913, *A.J.B. Chevalier 29659* [fr.] (P!); Ha Tinh Province, Vu Quang National Park, edge of lowland evergreen forest, roadside, 27 July 2015, *Nguyen N, Yahara T, Toyama H, Tagane S, Yang CJ, Tran DA V3822* [fr.] (DLU!, FU!, KYO!); Nghe An Province, Nui Chung, Kim Lien,

Nam Dan, 13 May 2016, *Nguyen V. N., Hoang T. B., V5369* [young fr.] (DLU!, FU!); ibid., Pumat National Park, Kem Waterfall, along the trail in the montane evergreen forest, 15 May 2016, *Nguyen V. N., Hoang T. B. V5449* (DLU!, FU!); Kon Tum Province, Ngoc Linh Nature Reserve, in lower montane evergreen forest, 15°11'39.2"N, 107°46'19.5"E, 1315 m alt., *Tagane S., Nagamasu H., Nguyen Van Ngoc, Hoang Thi Binh, Hoang Thanh Son, Yang C.-J., Kawakubo A. V6571* [fr.] (DLU!, FU!).

Distribution: Vietnam: Cao Bang, Lang Son, Ha Giang, Bac Giang, Nghe An, Ha Tinh, Kontum.

Lithocarpus bentramensis (A.Camus) A.Camus, Chênes Texte 3: 946 (1953).

Lithocarpus bacgiangensis subsp. *bentramensis* A.Camus, Chênes Atlas 3: 94 (1948).

TYPE: Vietnam, "Bên Trâm, prov. de Quang Tri", 15 March 1920, *Poilane 1123* (holotype: P [P00744507!]; isotype: P [P00744508!, P00744509!, P00744510!]).

Specimens examined: Vietnam, Quang Tri Province, "Bên Trâm, prov. de Quang Tri", 15 March 1920, *Poilane 1123* [fl., fr.] (P!).

Distribution: Vietnam: Quang Tri (Type locality), Dak Lak.

Lithocarpus chinhii Ngoc & Binh, **sp. nov.**

Fig. 4.3

Diagnosis: *Lithocarpus chinhii* is most similar to *L. bacgiangensis* but differs in having longer petioles, fewer secondary veins, obovoid or turbinate nut with mucronate apex and smaller basal scar (Table 4.3).

TYPE: Vietnam, Lam Dong Province, Bao Loc Pass, Roadside, 11°26'54.6"N, 107°43'19.6"E, alt. 588 m, 14 June 2015, *N. Nguyen, D. Luong, B. Hoang, T. Nguyen V3196* [fr.] (holotype: KYO!; isotype: DLU!, FU!, HN!, VNM!).

Description: Evergreen tree, up to 10 m tall. Twigs blackish gray, hairy. Leaves alternate; petiole 0.8–1.0 cm long, hairy; blade elliptic to lanceolate, 12–15 × 3.5–5 cm, coriaceous, apex acute or attenuate, base cuneate, margin entire, glabrous on both sides; midrib slightly prominent on both surfaces, yellowish *in vivo*, brownish yellow *in sicco*; secondary veins 12–13 pairs, slightly prominent abaxially, at an angle of 45–50° from the midrib; tertiary veins parallel, faintly visible to invisible on both sides. Inflorescences not seen. Infructescences terminal, erect, 12–17 cm long, 0.4 cm thick at base of axis, blackish brown, scurfy. Cupules usually clustered by 3, sessile to 0.2–0.3 cm stalked, saucer-shaped, 0.5–0.7 cm high, 1.2–1.4 cm in diam., enclosing 1/3–1/2 of the nut,

wall with very small triangular scales, scales not arranged into concentric rings, densely covered with whitish-brown indumentum. Nuts obovoid or turbinate, apex mucronate, 1.4–1.6 cm high, 1.1–1.3 cm in diam., densely hairy, reddish brown to grayish brown; basal scar concave, ca. 2 mm depth, 0.7–0.8 cm in diam.

Phenology: Fruiting specimens were collected in June.

Distribution and habitat: *Lithocarpus chinhii* is endemic to Lam Dong Province, southern Vietnam. We found only one fruiting individual along roadside and edge of evergreen forest, at 588 m altitude.

Etymology: The new species is named in honor of Prof. Nguyen Duy Chinh, Dalat University who had a significant contributed to the study of the Flora of Central Highland, Vietnam.

GenBank accession No. Ngoc et al. V3196: LC331652 (*rbcL*), LC331648 (*matK*), MG519665 (ITS).

Lithocarpus dinhensis (Hickel & A.Camus) A.Camus, Rivièra Sci. 18: 40 (1931).

Pasania dinhensis Hickel & A.Camus, Ann. Sci. Nat., Bot. 10, 3: 409 (1921); [P. H. Lecomte et al.] Fl. Indo-Chine 5: 1006 (1929).

TYPE: Vietnam, "In montibus Dinh ad Baria", March 1867, *Pierre 4969* (holotype: P [P04022511!]; isotypes: P [P00744697!, P00744698!, P00744699!]).

Specimens examined: Vietnam, Ba Ria-Vung Tau Province, "In montibus Dinh ad Baria", March 1867, *Pierre 4969* [fr.] (P!); Lam Dong Province, Bao Lam, B40 Pass, roadside and edge of evergreen forest, 11°44'03.3"N, 107°42'06.3"E, 850 m alt., 13 June 2015, *N. Nguyen, D. Luong, B. Hoang, T. Nguyen V3192* (DLU!, FU!); ibid., Bidoup-Nui Ba National Park, edge of evergreen forest, roadside, 19 June 2015, *N. Nguyen, D. Luong, B. Hoang V3241* [fr.] (DLU!, FU!).

Distribution: Vietnam: Quang Tri, Lam Dong (Langbian, Dran), Binh Phuoc (Dong Xoai), Ba Ria Vung Tau (Type locality). Laos, Cambodia.

Lithocarpus mekongensis (A.Camus) C.C.Huang & Y.T.Zhang, Guihaia 12: 2 (1992).

Lithocarpus microspermus subsp. *mekongensis* A.Camus, Chênes Atlas 3: 116 (1948).

TYPE: Laos. "Haut Mekhong: Entre Vien Poukha et Tafa", 800 m alt., 3 March 1936, *Poilane 26407* (holotype, P [P00744514!]).

Specimens examined: Laos, "Haut Mekhong: Entre Vien Poukha et Tafa", 800 m alt., 3 March 1936, *Poilane 26407* [fr.] (P!). Vietnam, Dang Nang, Mt. Son Tra, edge of evergreen, roadside,

16°06'58.72"N, 108°14'59.89"E, 249 m alt., 30 May 2015, *Ngoc Nguyen, H. Toyama, Chinh Nguyen V3125* [young fr.] (DLU!, FU!); ibid. 16°08'12.09"N, 108°14'02.13"E, 188 m alt., 30 May 2015, *Ngoc Nguyen, H. Toyama, Chinh Nguyen V3163* [young fr.] (DLU!, FU!); Kon Tum Province, Ngoc Linh Nature Reserve, in lower montane evergreen forest, 15°11'39.2"N, 107°46'19.5"E, 1315 m alt., 14 February 2017, *Tagane S., Nagamasu H., Nguyen Van Ngoc, Hoang Thi Binh, Hoang Thanh Son, Yang C.-J., Kawakubo A. V6581* [fl.] (DLU!, FU!); ibid. 15°12'24.2"N, 107°46'10.4"E, 1365 m alt., 14 February 2017, *Tagane S., Nagamasu H., Nguyen Van Ngoc, Hoang Thi Binh, Hoang Thanh Son, Yang C.-J., Kawakubo A. V6680* [young fr.] (DLU!, FU!).

Distribution: Laos (Type locality), Vietnam (Quang Tri, Da Nang).

Lithocarpus microspermus A.Camus, Bull. Soc. Bot. France 81: 818 (1934 publ. 1935).

TYPE: Vietnam, "Station agricole de Blao, province du Haut Donaï", 800 m alt., 4–26 April 1933, *Poilane 22355, 22259, 22413* (lectotype **designated here**: P [P00744688!]; isolectotype: P [P00744690!, P00744689!], VNM [VNM00020353!]).

Specimens examined: Vietnam, Lam Dong, "Station agricole de Blao, province du Haut Donaï", 800 m alt., 4 April 1933, *Poilane 22259* [fr.] (P!, VNM!); ibid., 24 April 1933, *Poilane 22355* [fr.] (P!, VNM!); ibid., 26 April 1933, *Poilane 22413* [fr.] (P!, VNM!); Lam Dong, Bao Lam, B40 Pass, roadside and edge of evergreen forest, 11°42'52.9"N, 107°43'54.3"E, 924 m alt., 13 June 2015, *N. Nguyen, D. Luong, B. Hoang, T. Nguyen V3174* [fr.] (DLU!, FU!); ibid., Di Linh, Gung Re, 11°28'23.5"N, 108°03'58.9"E, 1,100 m alt., 21 June 2015, *N. Nguyen, D. Luong, B. Hoang V3259* [young fr.] (DLU!, FU!); Quang Tri Province, 27 June 1930, *Poilane 13518* [young fr.] (VNM!). Thailand, Petchaburi Province, Kaeng Krachan, evergreen forest near Panoenthung ranger substation, 12°49'19.7" N, 99°21'57.7" E, 960 m alt., 24 October 2013, *Tagane S, Nagamasu H, Naiki A, Rueangruea S, Suddee S, Fuse K, Keiwbang W, Pansamrong P T2121* (BKF!, FU!).

Distribution: Vietnam: Lam Dong (Type locality), Quang Tri. Thailand.

Lithocarpus pierrei (Hickel & A.Camus) A.Camus, Rivièra Sci. 18: 41 (1931).

Pasania pierrei Hickel & A.Camus Ann. Sci. Nat., Bot. 10, 3: 398 (1921).

TYPE: Vietnam, "Austrochochinchinae: Ad Tou man in prov Bien hoa", March 1875, *Pierre 4979* (holotype: P [P00744551!]; isotype: P [P00744552!, P00744553!]).

Specimens examined: Vietnam, Dong Nai Province, "Austrochochinchinae: Ad Tou man in prov Bien hoa", March 1875, *Pierre 4979* [fr.] (P!); Lam Dong Province, Dalat, Prenn Pass, 11°53'57.6"N, 108°27'01.2"E, 1355 m alt., 14 June 2015, *N. Nguyen, D. Luong, B. Hoang, T. Nguyen V3199* (DLU!, FU!).

Distribution: Vietnam: Dong Nai (Type locality), Lam Dong

Litthocarpus pierreioides Ngoc, Tagane & Yahara, **sp. nov.**

Fig. 4.4

Diagnosis: *Lithocarpus pierreioides* is most similar to *L. pierrei* but differs in having smaller leaves, shorter petioles, fewer secondary veins, and smaller cupules and nuts (Table 4.3).

TYPE: Cambodia, Kampot Province, Phnom Bokor, evergreen forest margin, roadside, 10°36'18.46"N, 104°06'03.78"E, 530 m alt., 16 February 2013, *Tagane S, Toyama H, Wachi N, Ichihasi R, Mase K, Zhu M, Chhnang P 5514* [fr.] (holotype: KYO!, isotype: FU!, the herbarium of Forest Administration on Cambodia).

Description: Evergreen tree, up to 10 m tall. Twigs blackish gray, glabrous. Leaves alternate; petiole 0.8–1.0 cm long, glabrous; blade oblong or lanceolate, 10.5–14 × 3–4.5 cm, coriaceous, apex acute or attenuate with acumen ca. 1.0 cm long, base cuneate, margin entire, glabrous on both sides; midrib slightly prominent beneath, yellowish *in vivo*, reddish brown *in sicco*; secondary veins 12–13 pairs, slightly prominent abaxially, at an angle of 45° from the midrib, tertiary veins parallel, faintly visible to invisible on both sides. Inflorescences not seen. Infructescences terminal, erect, 12–18 cm long, 0.6 cm thick at base of axis, grayish brown *in sicco*, covered with very short indumentum. Cupules solitary, rarely clustered by 2 or 3, almost sessile stalked, saucer-shaped, 0.5–0.7 cm high, 0.9–1.1 cm in diam., enclosing 1/2 or more of the nut; wall with very small triangular scales, imbricate, densely grayish indumentum. Nuts conical shaped, apex acuminate, 0.5–0.7 cm high, 0.8–1 cm in diam., covered with densely grayish indumentum, reddish brown to grayish brown when dry; basal scar flat or slightly convex, ca. 0.5 cm in diam.

Phenology: Fruiting specimens were collected in February.

Distribution and habitats: Cambodia, so far known only from type locality, Mt. Bokor, in the evergreen forest, ca. 500 m altitude.

*Distribution***:** Cambodia. So far known only from type locality, Mt. Bokor, evergreen forest.

Etymology: The species epithet "*pierreioides"* is derived from its morphological traits most similarity to *L. pierrei.*

GenBank accession No. Tagane et al. C5514: LC331651 (*rbcL*), LC331647 (*matK*), MG519664 (ITS).

Lithocarpus pseudoannamensis Ngoc & Binh, **sp. nov.**

Fig. 4.5

Diagnosis: *Lithocarpus pseudoannamensis* is most similar to *L. annamensis* but differs in having bigger leaf size (15–23.5 cm × 4.5–8.5 cm vs. 13–15 cm × 4.5–5.5 cm), more secondary veins (12–14 pairs vs. 11–12 pairs), longer infructescences (12–17 cm long vs. 5–10 cm long), cupule covering 1/3–1/2 of the nut (vs. only base of the nut) and bigger basal scar of nut (10–12 mm in diam. vs. 7 mm in diam.) (Table 4.3).

TYPE: Vietnam, Lam Dong Province, Di Linh, Gung Re, Roadside, 11°28'23.5"N, 108°03'58.9"E, alt. 1,100 m, 21 June 2015, *N. Nguyen, D. Luong, B. Hoang V3261* [fr.] (holotype: KYO!, isotype: DLU!, FU!, HN!, VNM!).

Description: Evergreen tree, up to 10 m tall. Twigs blackish gray when dry, hairy. Leaves alternate; petiole 0.8–1.4 cm long, hairy; blade elliptic to lanceolate, 14–23.5 × 4.5–8.5 cm, coriaceous, apex acute or attenuate, base cuneate or obtuse, margin entire, glabrous on both sides; midrib prominent on both surfaces, yellowish *in vivo*, brownish yellow *in sicco*; secondary veins 12–14 pairs, slightly prominent abaxially, at an angle of 40–50° from the midrib; tertiary veins parallel, faintly visible on both sides. Inflorescences not seen. Infructescences terminal, erect, 12–17 cm long; axis grayish brown, scurfy, covered with very short indumentum, 0.4–0.6 cm thick at base. Cupules usually clustered by 3, sessile to 3 mm stalked, saucer-shaped, 0.5–0.8 cm high, 1.7–2.2 cm in diam., enclosing 1/3–1/2 of the nut; wall with small triangular scales not united into concentric rings, densely covered with grayish indumentum. Nuts strongly depressed oblate, 1.3–1.6 cm high, 1.6–2 cm in diam., densely covered with tawny minute hairs, reddish brown to grayish brown; basal scar slightly concave, ca. 1–1.2 cm in diam.

Additional specimens examined: Vietnam, Lam Dong Province, Di Linh, Gung Re, Roadside, 11°28'23.5"N, 108°03'58.9"E, alt. 1,100 m, 21 June 2015, N. Nguyen, D. Luong, B. Hoang V3260 [fr.] (DLU!, FU!).

*Phenology***:** Fruiting specimens were collected in June.

Distribution and habitats: Vietnam (so far known only from Gung Re Pass, Di Linh District, Lam Dong Province). In lower montane evergreen forest, ca. 1000 m altitude.

Etymology: The species epithet "*pseudoannamensis"* is derived from its morphological traits look like *L. annamensis.*

Genbank accession No. Ngoc et al. V3261: LC331654 (*rbcL*), LC331650 (*matK*), MG519667 (ITS).

Lithocarpus pseudovestitus A. Camus, Bull. Soc. Bot. France 86: 155 (1939).

TYPE: Vietnam, "Tonkin: Massif de Nui Bien, près Chobo", 1,100 m alt., 2 September 1926, *Poilane 13106* (syntypes: P [P00744581!, P00744582!]).

Specimens examined: Vietnam, Hoa Binh Province, "Tonkin: Massif de Nui Bien, près Chobo", 1100 m alt., 2 September 1926, *Poilane 13106* [fr.] (P!); Vinh Phuc, Vinh Yen, Tam Dao, 8 September 1931, *Casalta 8* [fr.] (P!); Ha Tinh Province, Vu Quang National Park, 18°15'38.3"N, 105°20'49.6"E, 1,079 m alt., 26 July 2015, *Nguyen N, Yahara T, Toyama H, Tagane S, Yang CJ, Nguyen H V3795* (DLU!, FU!); ibid., 18°12'20.0"N, 105°23'23.3"E, 1,335 m alt., 24 June 2016, *Yahara T., Nguyen V.N., Toyama H., Tagane S., Okabe N., Nguyen V.H. V5930* (DLU! FU! The herbarium of Vu Quang National Park!).

Distribution: Vietnam: Hoa Binh (Type locality), Vinh Phuc, Ha Tinh.

Lithocarpus vestitus (Hickel & A.Camus) A.Camus, Rivièra Sci. 18: 42 (1931).

Pasania vestita Hickel & A.Camus, Ann. Sci. Nat., Bot. 10, 3: 393 (1921); [P. H. Lecomte et al.] Fl. Indo-Chine 5: 980 (1929).

TYPE: Vietnam, Khanh Hoa Province, Nha Trang, *Krempf 1913* (lectotype **designated here**: Camus (1948) Plate 454!).

Specimens examined: Vietnam, Khanh Hoa Province, "massif de Cô Inh pres Nhatrang", 700 m alt., 18 September 1922, *Poilane 4640* [fr.] (VNM!); ibid., Mt. Hon Ba, in transect line 4, placed in slope of evergreen forest, 12° 6'39.77"N, 108°58'59.23"E, 617 m alt., 22 February 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. V1139* [fr.] (FU!, VNM!, The herbarium of Hon Ba Nature Reserve!).

Distribution: Vietnam: Khanh Hoa (Type locality).

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Legends

Table 4.1. List of our specimens used in this study.

Species	Vouchers	Localities
Lithocarpus bacgiangensis	*Nguyen et al. V3822* (DLU, FU)	Vu Quang NP
	Nguyen et al. V5369, V5449 (DLU, FU)	Pu Mat NP, Nghe An Province
	Tagane et al. V6571 (DLU, FU)	
Lithocarpus chinhii	*Nguyen et al. V3196* (DLU, FU)	Bao Loc, Lam Dong Province
Lithocarpus dinhensis	*Nguyen et al. V3192, V3241* (DLU, FU, VNM)	Bidoup-Nui Ba NP
Lithocarpus mekongensis	*Ngoc et al. V3125* (DLU, FU)	Son Tra, Da Nang
	Ngoc et al. V3163 (DLU, FU)	
	Tagane et al. V6581, V6680 (DLU, FU)	Ngoc Linh NR
Lithocarpus microspermus	*Tagane et al. T2121* (FU)	Kaeng Krachan NP, Thailand
	Ngoc et al. V3174, V3259 (DLU, FU)	Bidoup-Nui Ba NP
Lithocarpus pierrei	*Nguyen et al. V3199* (DLU, FU)	Bidoup-Nui Ba NP
Lithocarpus pierreioides	*Tagane et al. C5514* (FU)	Phnom Bokor NP
Lithocarpus pseudoannamensis	*Nguyen et al. V3260, V3261* (DLU, FU)	Di Linh, Lam Dong Province
Lithocarpus pseudovestitus	*Nguyen et al. V3795* (DLU, FU)	Vu Quang NP
	Yahara et al. V5930 (DLU, FU)	
Lithocarpus vestitus	*Toyama et al. V1139* (FU, VNM)	Hon Ba NR
Trigonobalanus verticillata	*Yahara et al. V5764* (FU, DLU)	Vu Quang NP

Table 4.2. Primer set for classic DNA barcoding

Region	Primer	Direction	Sequences 5' → 3'	Reference
rbcL	r*bcL*a-F	F	ATGTCACCACAAACAGAGACTAAAGC	Levin et al. (2003)
	rbcL-724R	R	TCGCATGTACCTGCAGTAGC	Fay et al. (1997)
matK	*matK*3_F_XF	F	TAATTTACGATCAATTCATTC	Ford et al. (2009)
	*matK*18_R_1329R	R	TCTAGCACACGAAAGTCGAAGT	Cuénoud et al. (2002)
ITS	ITS-18F	F	GTCCACTGAACCTTATCATTTAGAGG	Rohwer et al. (2009)
	ITS-26R	R	GCCGTTACTAAGGGAATCCTTGTTAG	

F, Forward; R, Reverse.

Table 4.3. The morphological comparison of new taxon with its relative species.

Characters	*L. annamensis*[1,2]	*L. pseudoannamensis*	*L. bacgiangensis*[1,2,3]	*L. chinhii*	*L. pierrei*[1,2,3]	*L. pierreioides*
Leaf margin	Entire	Entire	Entire	Entire	Entire	Entire
Leaf size	13–15 cm long × 4.5–5.5 cm wide	15–23.5 × 4.5–8.5 cm	14–15 cm long × 5 cm wide	12–15 cm long × 3.5–5 cm wide	25–30 cm long × 6–8 cm wide	10.5–14 cm × 3–4.5 cm
Petioles	0.5–1.5 cm long	0.8–1.4 cm long	0.5–0.7 cm long	0.8–1 cm long	1.5 cm long	0.8–1 cm long
Number of secondary veins	11–12 pairs	12–14 pairs	14–15 pairs	12–13 pairs	15–16 pairs	12–13 pairs
Infructescences length	5–10 cm long	12–17 cm long	10–20 cm long	12–17 cm long	10–20 cm long	12–18 cm long
Fruiting stalk	Almost sessile	Sessile to 3 mm long	Almost sessile	Almost sessile	Almost sessile	Almost sessile
Cupule	Clustered by 3, plate-shape	Clustered by 3, bowl-shaped	Clustered by 3, bowl-shaped	Clustered by 3, bowl-shaped	Solitary or clustered by 2–3, plated-shaped	Solitary or clustered by 2–3, plated-shaped
Cupule size	14–15 mm in diam.	5–8 mm high × 17–22 mm in diam.	5 mm high × 15 mm in diam.	5–7 mm high × 12–14 mm in diam.	5–7 mm × 15–17 mm in diam.	5–7 mm high × 9–11 mm diam.
Cupule surfaces	Dense whitish gray indumentum	Sense whitish gray indumentum	Dense whitish gray indumentum	Dense whitish gray indumentum	Tomentose	Dense grayish indumentum
Scales arrangement	Scales not united into concentric rings	Scales not united into concentric rings	Imbricate	Very small triangular scales, imbricate	Very small triangular scales, imbricate	Very small triangular scales, imbricate
Nut shape and surface	Depressed globose	Strongly depressed oblate	Obovoid or turbinate, apex depressed or flat	Obovoid or turbinate, mucronata	Subglobosa, mucronata	Conical
Nut size	10–13 mm high × 17–22 mm in diam.	13–16 mm high, 16–18 mm in diam.	11–12 mm high × 17–18 mm in diam.	14–16 mm high × 11–13 mm in diam.	7–8 mm high × 15–17 mm in diam.	5–7 mm high, 8–10 m in diam.
Nut enclosure	Base of nut	Enclosing 1/3–1/2 of the nut	1/3–1/2 of the nut	1/3–1/2 of the nut	1/2 or more	1/2 or more
Scar of the nut	Concave, 7 mm in diam.	Slightly concave, ca. 10–12 mm in diam.	Concave, 10–12 mm in diam.	Concave, ca. 7–8 mm in diam.	Subconcave	Flat or slightly convex, ca. 5 mm in diam.

[1] Derived from original description; [2] Derived from type materials and herbaria collections, [3] Derived from this study collections

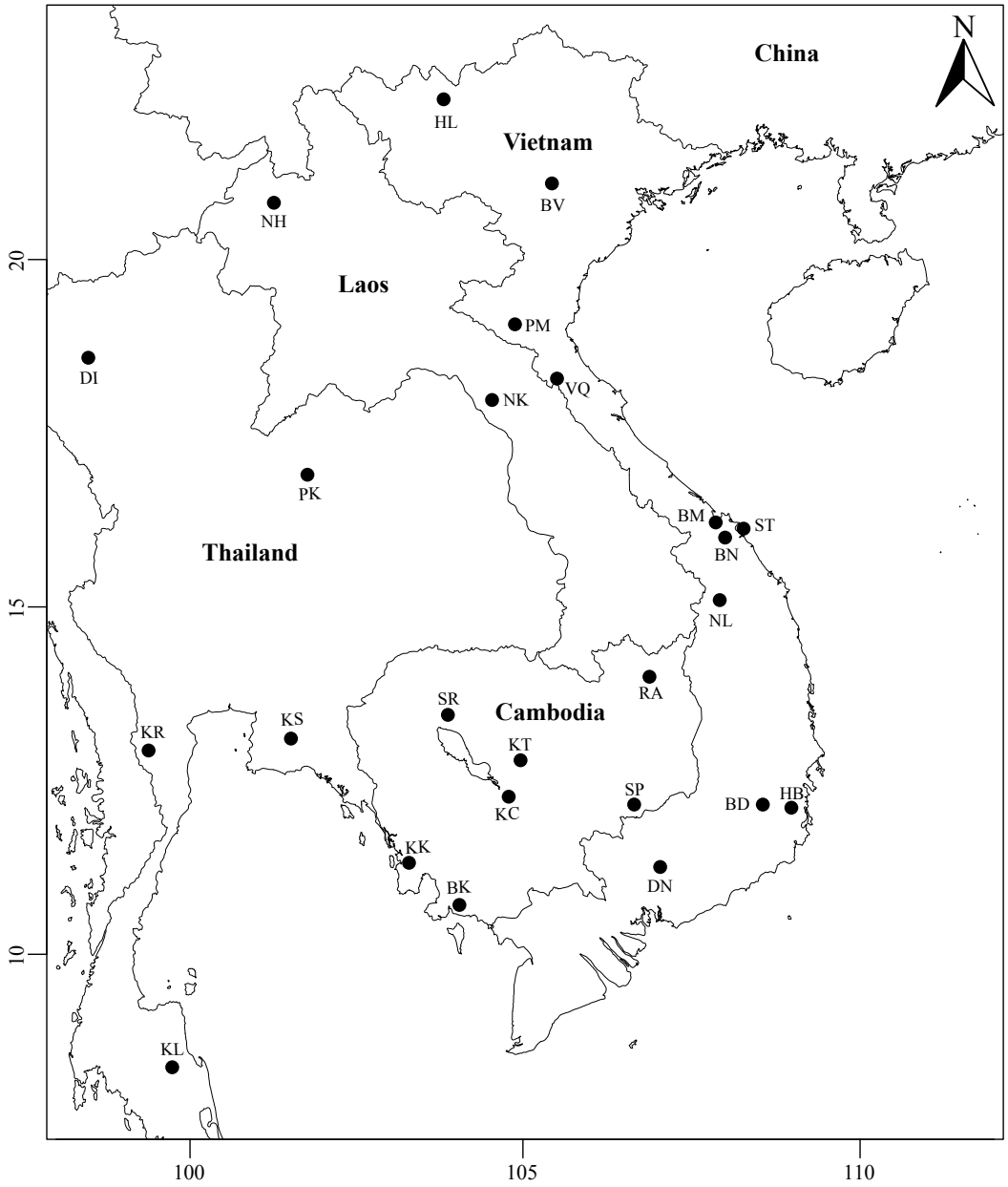

Figure 4.1. Collection sites in Vietnam, Laos, Cambodia and Thailand in this study: (**HL**) Hoang Lien NP, (**BV**) Ba Vi NP, (**PM**) Pu Mat NP, (**VQ**) Vu Quang NP, (**BM**) Bach Ma NP, (**BN**) Ba Na NR, (**ST**) Son Tra Peninsula, (**NL**) Ngoc Linh NR, (**HB**) Hon Ba NR, (**BD**) Bidoup-Nui Ba NP, (**DN**) Dong Nai NR, (**NH**) Nam Ha NP, (**NK**) Nam Kading NP, (**SR**) Siem Reap, (**RA**) Ratanakiri, (**KT**) Kampong Thom, (**SP**) Seima Protected Forest, (**KC**) Kampong Chhnang, (**KK**) Koh Kong, (**BK**) Bokor, (**DI**) Doi Inthanon NP, (**PK**) Phukradueng NP, (**KS**) Khao Soi Dao Wildlife Sanctuary, (**KR**) Kaeng Krachan NP, (**KL**) Khao Luang NP. The map was generated and modified by Nguyen Van Ngoc using RStudio ver. 1.1.383 (mapdata packages, RStudio, Inc.) and Adobe Illustrator CC 2017 ver. 21.0.0 (Adobe Systems, San Francisco, CA, USA).

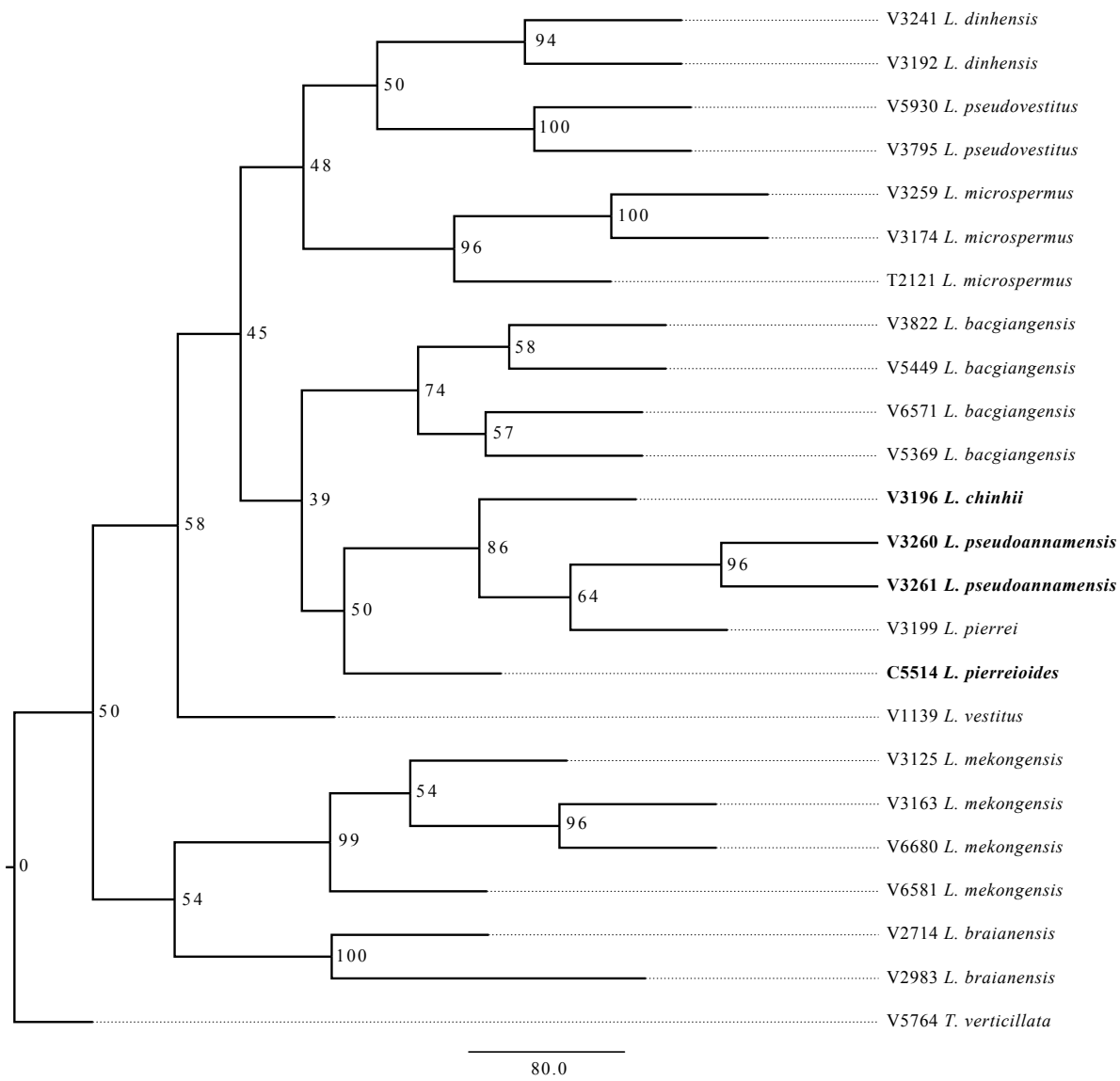

Figure 4.2. NJ tree of 23 samples of *Lithocarpus* and one *Trigonobalanus* (outgroup) based on presence/absence data of 18,226 MIG-seq loci. Branches are labeled with bootstrap support (% of 1000 replicates). Bold indicates new species described in this study.

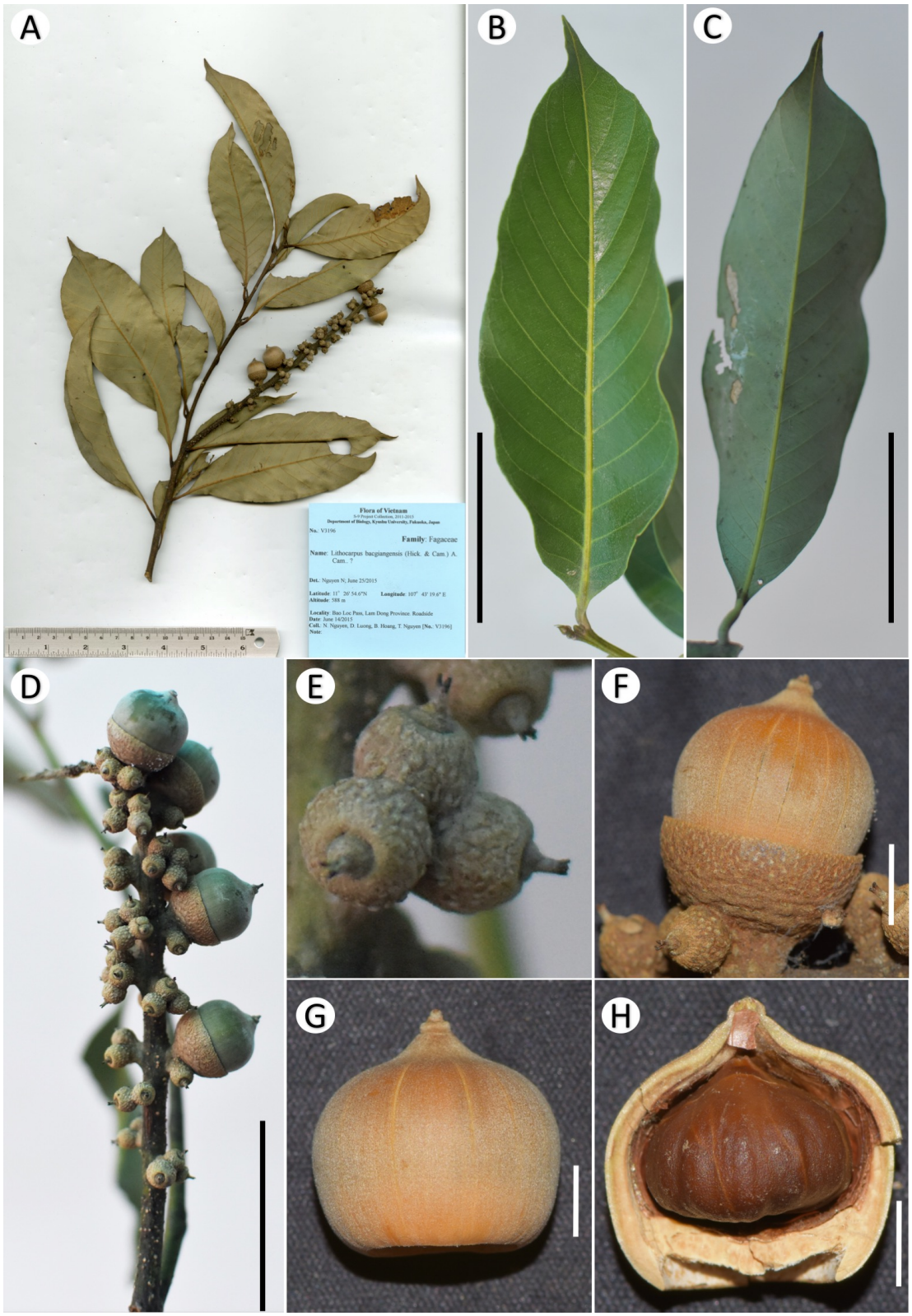

Figure 4.3. *Lithocarpus chinhii* Ngoc & Binh: (**A**) Holotype (KYO), (**B**) Abaxial leaf surface, (**C**) Adaxial leaf surface, (**D**) Infructescences, (**E**) Young clustered acorn, (**F**) Mature acorn, (**G**) Mature nut, (**H**) Vertical sections of nut. Scale bars (**B–D**) = 5 cm, (**F–H**) = 5 mm. Materials from *Ngoc et al. V3196.*

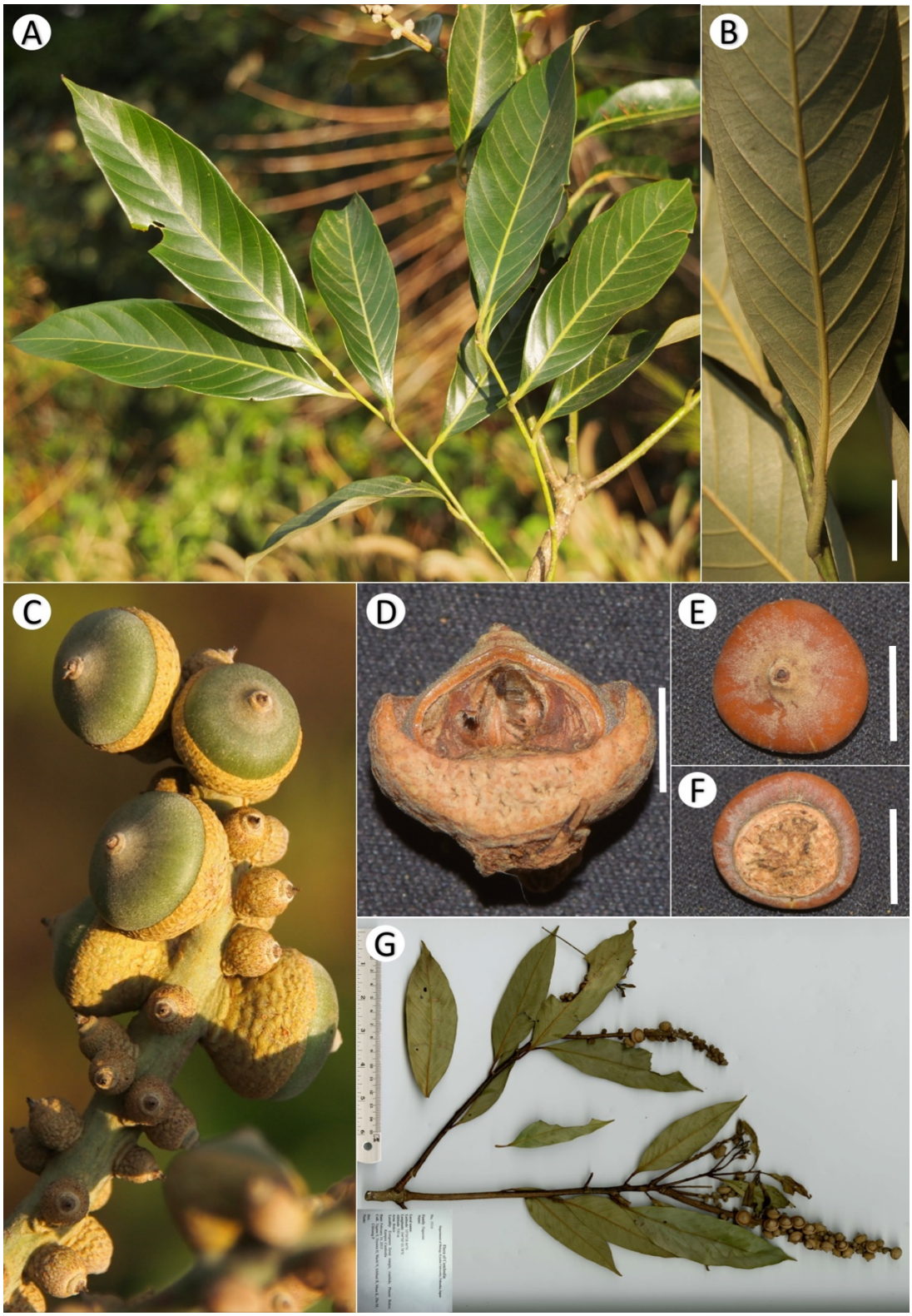

Figure 4.4. *Lithocarpus pierreioides* Ngoc, Tagane & Yahara: (**A**) Leafy twig, (**B**) Abaxial leaf surface, (**C**) Infructescence, (**D**) Vertical section of the fruit, (**E**) Mature nut (**F**) Nut scar, (**G**) Holotype (KYO). Scale bars (**B**) = 1 cm, (**D–F**) = 5 mm. Materials from *Tagane et al. 5514.*

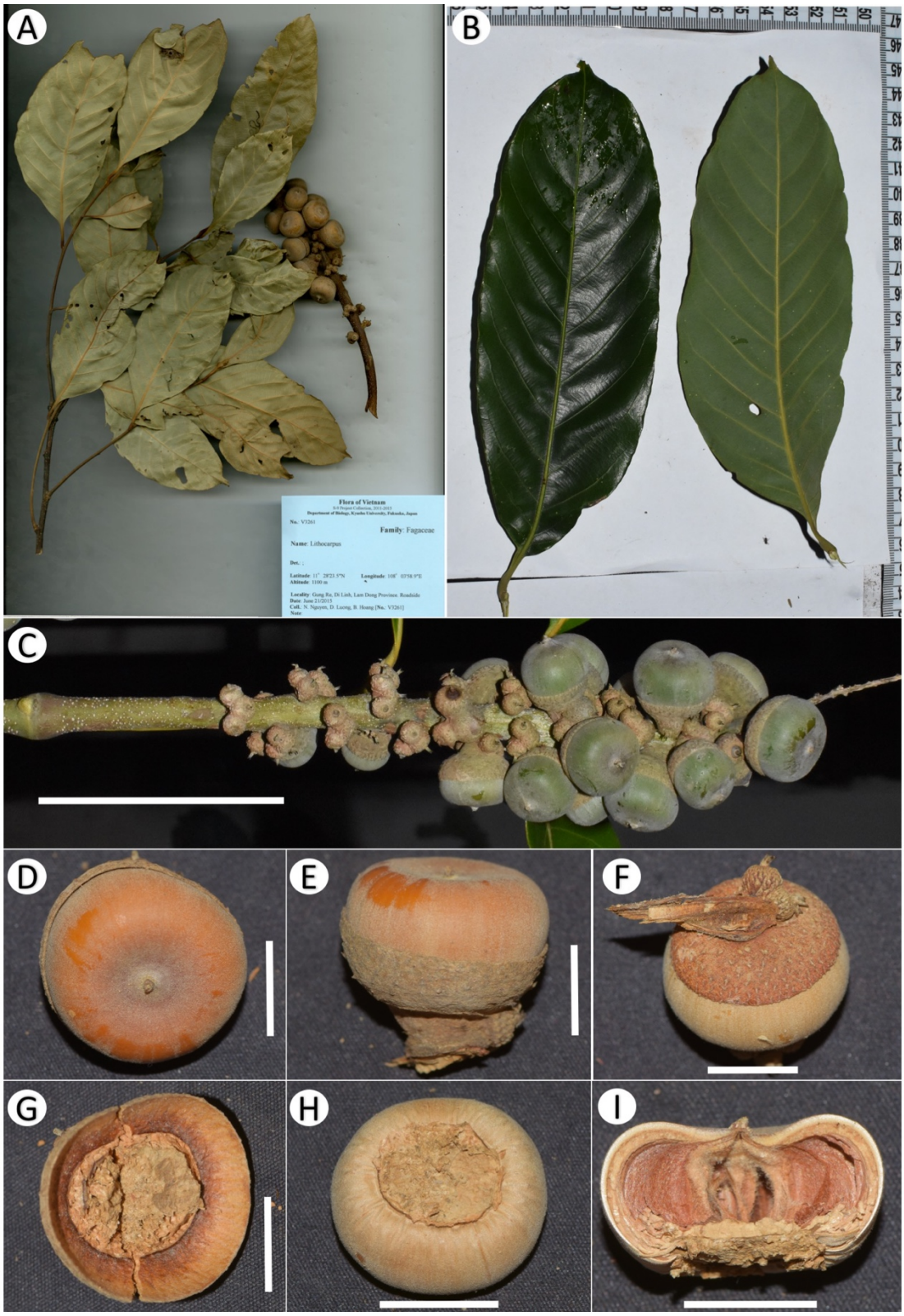

Figure 4.5. *Lithocarpus pseudoannamensis* Ngoc & Binh: (**A**) Holotype (KYO), (**B**) Abaxial & adaxial leaf surface, (**C**) Infructescences, (**D-F**) Top view, side view & bottom view of the fruits, (**G**) Inside of cupule, (**H**) Nut scar, (**I**) Vertical section of the nut. Scale bars (**C**) = 5 cm, (**D–I**) = 1 cm. Materials from *Ngoc et al. V3261*.

Chapter V
A taxonomic study of *Lithocarpus elegans*, *L. hancei* and its relatives (Fagaceae) in Southeast Asia, based on next generation DNA barcodes and morphological observations

Abstract

To revise the taxonomy of *Lithocarpus elegans* complex, *L. hancei* complex and their close relatives in Vietnam, we made a molecular phylogenetic analysis based on multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) on 72 samples of *Lithocarpus* spp. collected mostly from Vietnam and partly from Cambodia, Indonesia, Myanmar and Thailand. The evidence obtained from this analysis and subsequent morphological observations on 72 samples as well as herbarium specimens including types showed that *L. elegans s. lat.* is not a single widespread species but a group of cryptic species including *L. blaoensis, bokorensis,* sp. nov., *L. elegans s. str., L. monoromensis,* sp. nov., *L. grandifolius,* and *L. syncarpus*. A morphologically distinct species *L. harmandii* is sister to *L. bokorensis* in the MIG-seq tree. *Lithocarpus blaoensis* treated as a synonym of *L. elegans* is not close to other species of the *L. elegans* complex but sister to *L. petelotii*. In *Lithocarpus hancei* complex, the MIG-seq tree supported distinctiveness of three species, *L. hancei*, *L. jacksonianus* and *L. yersinii*. In vegetative morphology, *L. hancei* and *L. jacksonianus* were indistinguishable but both occur in the same locality of northern Vietnam, supporting that those are two cryptic species. *Lithocarpus hancei* is sister not to *L. jacksonianus* but to *L. yersinii* distributed in southern Vietnam. In conclusion, six species of the *L. elegans* complex and three species of the *L. hancei* complex are recognized.

Key words

DNA barcodes, Fagaceae, *Lithocarpus,* new species, taxonomy, Vietnam.

Introduction

The family Fagaceae Dumort. is widely distributed mainly in the Northern Hemisphere and often dominant in temperate, subtropical as well as tropical forests. The family is often divided into two or three subfamilies (Forman 1966, Hutchinson 1967, Nixon & Crepet 1989) under which nine genera with ca. 1000 species are generally accepted (Manos et al. 2001). Diversity of the genus is the highest in East and Southeast Asia, where six genera are represented including *Castanea* Mill., *Castanopsis* (D.Don.) Spach., *Fagus* L., *Lithocarpus* Blume, *Quercus* L. and *Trigonobalanus* Forman (Soepadmo 1972). Diversity at the species level is also very high in East and Southeast Asia where *Castanopsis* and *Lithocarpus* are particularly diverged.

Lithocarpus is the second largest genus of the family Fagaceae, comprising more than 300 species (Camus 1954, The Plant list 2013). Its species diversity is the highest in China and Vietnam where 123 species (Huang et al. 1999) and 117 species (Ban 2003, Ho 2003) were reported, whereas 31 species (Kress et al. 2003), 41 species (Newman et al. 2007) and 56 species (Phengklai 2008) were recorded in Myanmar, Laos and Thailand, respectively. Among Chinese species, 32 species had been described since 1960 by Chinese taxonomists. On the other hand, Vietnamese species were mostly described by French botanists until 1920s and some others were until 1950s. Since then, the taxonomic inventory of *Lithocarpus* in Vietnam had a long blank until Ngoc et al. (2016) described a new species based on their newly collected materials. More recently, Ngoc et al. (in review) employed molecular approaches to revise the *Lithocarpus vestitus* complex and described three additional new species. These recent findings suggest that a considerable number of species would still remain to be described in Vietnam.

Here, we apply the molecular approaches employed by Ngoc et al. (in review) to *Lithocarpus elegans* (Blume) Hatusima ex Soepadomo, *L. hancei* (Benth.) Rehder and their relatives. In Vietnam and its adjacent countries, there is a group of species having relatively large and thick, basally cuneate, and glabrous and greenish leaves. In those species, some species show a set of three cupules usually deeply connate when mature. We designate these species as *L. elegans* complex hereafter. *Lithocarpus hancei* also has thick and glabrous leaves greenish on both surfaces, but leaves are relatively smaller and a set of three cupules are mostly isolated or solitary when mature. We designate *L. hancei* and its morphologically similar species as *L. hancei* complex hereafter.

Among the species recorded in Flore Générale de I'Indo-Chine (Hickel & Camus 1929), *Pasania spicata* (Sm.) Oerst. is a member of the *Lithocarpus elegans* complex, and is treated as a synonym of *L. elegans* by Soepadmo (1972), Ho (2003) and Phengklai (2008) or as *L. grandifolius* (D.Don) Biswas. by Huang et al. (1999). After Hickel & Camus (1929), two species of the *L. elegans* complex, *L. blaoensis* (A.Camus) A.Camus (Camus 1935) and *L. syncarpus* A.Camus (Camus 1939) were described. However, Ho (2003) treated these two as synonyms of *L. elegans*. Phengklai (2008) treated *L. grandifolius* described from India as a synonym of *L. elegans*. Therefore, the relationship of *L. elegans* and its relatives is still controversial. Hence the first purpose of this study is to examine whether *L. elegans* in the broad sense of Ho (2003) and Phengklai (2008) is really a single widespread species or composed of some cryptic species. In addition, we also examine the relationship of the *L. elegans* complex and *L. harmandii* (Hickel & A.Camus) A.Camus with hairy leaves because our molecular study suggested that *L. harmandii* is placed within a clade corresponding to the *L. elegans* complex.

The *L. hancei* complex includes *L. hancei* described from China (Bentham 1861), *L. sabulicolus* (Hickel & A.Camus) A.Camus and *L. jacksonianus* A.Camus from central Vietnam (Hickel & Camus 1929, Camus 1945) and *L. yersinii* A.Camus from southern Vietnam (Camus 1934). The distribution ranges of those morphologically similar species are mostly allopatric, and it remains uncertain whether those are distinct species or geographical races within a single widespread species.

To answer the above two questions for the *L elegans* complex and the *L. hancei* complex, we made molecular phylogenetic analyses and morphological observations based on samples recently collected in a series of our field surveys in Cambodia, Indonesia, Laos, Myanmar, Thailand, and Vietnam, (Yahara et al. 2012, Zhang et al. 2016, Ngoc et al., in review). The results showed that (1) three lineages of the *L. elegans* complex are nested with two lineages of "*L. harmandii*" and those five lineages are to be considered as distinct species, and (2) the above four species of the *L. hancei* complex are distinct species. Based on this conclusion, we describe two new species of *Lithocarpus*, *L. bokorensis*, and *L. monoromensis* from Cambodia.

Materials and methods

Study site and sample collection

From 2011 to present, we carried out field surveys and collected specimens of Fagaceae as a part of the plant diversity assessment project in Southeast Asia. We used standardized rectangular plots of 100 m × 5 m (Yahara et al. 2012, Zhang et al. 2016) placed at various locations along the altitudinal and latitudinal gradient to record and collect plant specimens in the field. In addition, we made general collections around the study sites. We visited 140 sites in the 35 locations of Southeast Asia, and collected 498

specimens of *Lithocarpus* including 315 specimens from Vietnam, 54 from Thailand, 48 from Indonesia, 45 from Cambodia, 24 from Laos, and 12 from Myanmar (Fig. 5.1). After preliminary studies of morphology and molecular divergence on those specimens, we selected 25 specimens of the *L. elegans* complex, 4 specimens of *L. harmandii* subsp. *harmandii* and *L. harmandii* (A.Camus) subsp. *malacotrichus* A.Camus that are related to the *L. elegans* complex, and 19 specimens of the *L. hancei* complex for further detail analyses. To confirm the reliability of topology of the phylogenetic tree, 3 samples of *L. petelotii* A.Camus, one sample of *L. pleiocarpus* A.Camus, 4 samples of *L. litseifolius* (Hance) Chun, 7 samples of *L. rouletii* (Hickel & A.Camus) A.Camus, and 9 samples of *L. balansae* (Drake) A.Camus were also added for the phylogenetic analysis. Two species of *Castanopsis* and three species of *Quercus* were included in the phylogenetic analysis as outgroups. The information of specimens used in the present study were provided in the Supplementary 5.S1.

DNA extraction

Total DNA was extracted from silica-gel dried leaf fragments by the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987) with minor modifications described in Toyama et al. (2015). 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).

Multiplexed ISSR genotyping by sequencing (MIG-seq)

Multiplexed ISSR genotyping by sequencing (MIG-seq; Suyama and Matsuki 2015) was employed to obtain molecular data for phylogenetic and population genetic

studies of species in the *L. elegans* complex and the *L. hancei* complex. MIG-seq library was prepared following Suyama and Matsuki (2015) except for using dual indexed primers. Approximately 10 ng/μl Genomic DNA was used as templates for the first PCR, multiple non-repetitive regions from various inter-simple-sequence repeats (ISSRs) were amplified from genomic DNA by multiplexed PCR with tailed ISSR primers. The diluted products were used as the templates for the 2nd PCR (tailed PCR). Then, 3 μl of each 2nd PCR product was pooled in equimolar concentration, and fragments in the size range of 350–800 bp were isolated. After the measurement of the final concentration, 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). Materials of the following species were not included in the MIG-seq analysis; *L. elegans s. str.* of the *L. elegans* complex and *L. sabulicolus* of the *L. hancei* complex.

SNP detection

Fastx-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) and TagDust program (Lassmann et al. 2009) were used to remove the primer regions and control quality of the raw data as described in Suyama and Matsuki (2015). Then, the quality-filtered data was used for SNP detection using Stacks ver. 1.35 (Catchen et al. 2011). First, assembled homologous sequences (loci) in each individual using the 'ustacks' option of Stacks, with the following settings: maximum distance between stack (M) = 1, maximum distance allowed to align secondary reads to primary stacks (N) = 1, the deleveraging algorithm (d), the removal algorithm (r) and gapped alignment option (gapped) enabled. The minimum depth of coverage required to create a stack (m) is set as 10. Second, a catalog was created for all possible loci and alleles with the 'cstacks' option. The parameter 'number of allowed mismatches between samples (n)' was set as four. All stacks created

by ‘ustacks’ were then matched against the catalog produced by ‘cstacks’, using the ‘sstacks’ option.

Selection of SNP markers.

We extracted SNPs from the filtered data set of each complex using the population option of Stacks by selecting the ‘write_single_snp’ option with the following setting: (1) minimum of samples with a SNP in a population (r) = 0.75%, (2) minimum number of populations in a locus (p) = 1, (3) minimum minor allele frequency (min_maf) = 0.05, and (4) maximum observed heterozygosity (max_obs_het) = 0.95.

For population genetic analyses, two sub-datasets (*L. syncarpus* group and *L. harmandii* group) of the *L. elegans* complex and one sub-dataset of the *L. hancei* complex were extracted to determine the genetic structure of these species groups. The population option of Stacks was used with the same parameter setting as described above to create the structure input data.

Phylogenetic analysis

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”. The presence/absence (1/0) data of loci were used to computed distance matrix, constructed a neighbor-joining (NJ) tree, and examined the reliability of tree topology by bootstrapping with 1000 replicate using PHYLYP ver. 3.695 (Felsenstein 2005) as follows. First, we used Seqboot for 1000 times resampling from presence/absence data of loci. Second, distance matrices were computed with Restdist program. Third, the distance matrices are used to infer the neighbor-joining (NJ) trees. Finally, a consensus tree was constructed

with Consense. The resulted tree was visualized with FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Morphological and taxonomic comparison

Morphological traits of each species of the complex as well as its related species were carefully reexamined considering the result of phylogenetic and population genetic analyses and using our specimens as well as specimens kept in the herbaria ANDA, BKF, DLU, HN, KYO, P, and VNM and digitized plant specimen images available on the web of JSTOR Global Plants (https://plants.jstor.org/) and Chinese Virtual Herbarium (http://www.cvh.org.cn/). We identified species using the type specimens, original descriptions and diagnostic traits described in taxonomic literature (Soepadmo 1972, Ho 1999, Huang 1999, Ban 2005, Phengklai 2008).

Population genetic structure analysis

For a clade including more than two specimens, we created a sub-dataset and conducted a population genetic analysis using STRUCTURE ver. 2.3.3 (Pritchard et al. 2000). The aim of this analysis was to confirm whether those individuals belong to single mixing population or some clusters of highly isolated subpopulations. Assuming that all specimens are in the same population (p = 1), the number of clusters (*K*) from 1 to 9 were tested by running 20 simulations for each *K*, with 50,000 Markov chain Monte Carlo iterations following a burn-in period of 50,000, using the model with admixture and correlated allele frequencies. STRUCTURE HARVESTER (Earl & vonHoldt 2012) was used for visualizing outputs of STRUCTURE analyses and implementing the Evanno method to determine the optimal value of *K*. The replicate runs were combined by

CLUMPP (Jakobsson & Rosenberg 2007) and the results were visualized using STRUCTURE PLOT program (Ramasamy et al. 2014).

Results

Phylogenetic inference

In the Neighbor joining (NJ) tree based on presence/absence data of 34,449 MIG-seq loci (Fig. 5.2), a clade (Clade 1) consisting of *L. balansae* and *L. rouletii* was placed as an outgroup of another clade (Clade 2) including the species of *L. elegans* and *L. hancei* complexes. Clade 2 was diverged to three clades; Clade 2A, Clade 2B and Clade 2C supported by 98 %, 90 % and 98% bootstrap values, respectively, among which Clade 2A and Clade 2B were sister to each other and Clade 2C (*L. litseifolius*) was placed as an outgroup of Clade 2A and Clade 2B. In Clade 2A, four species of *L. elegans* complex (*L. syncarpus*, *L. bokorensis*, *L. monoromensis* and *L. grandifolius*) were clustered with two hairy species, *L. harmandii* subsp. *harmandii* and *L. harmandii* subsp. *malacotrichus*, whereas three species of *L. hancei* complex (*L. hancei*, *L. jacksonianus* and *L. yersinii*) and a species of *L. elegans* complex (*L. blaoensis*) were included in Clade 2B in which a clade including three species of *L. hancei* complex was sister to another clade including *L. blaoensis, L. petelotii and L. pleiocarpus*.

For *L. elegans* complex, *L. grandifolius* (MY1552) was placed in the basal branch of Clade 2A and the other species were clustered to three well-supported (BP=100%) clades, Clades 2A1 (*L. syncarpus*), 2A2 (*L. bokorensis*, *L. harmandii* subsp. *harmandii* and *L. harmandii* subsp. *malacotrichus*) and 2A3 (*L. monoromensis*). In Clade 2A2, *L. bokoensis* was sister to a clade consisting of *L. harmandii* subsp. *harmandii* and *L. harmandii* subsp. *malacotrichus*.

For *L. hancei* complex, *L. jacksonianus*, *L. yersinii* and *L. hancei* were clustered to Clade 2B1, 2B2 and 2B3 supported by 76%, 86% and 93% BP, respectively, and aff. *L. hancei* (V5111) and aff. *L. yersinii* (V65) were placed in the basal branches of Clade 2B1. Monophyly of the nine samples of *L. jacksonianus* was supported by 94% BP.

Morphological observation (Table 5.1 & 5.2)

Lithocarpus blaoensis (V3176) is similar to *L. grandifolius* (MY1552) not only in the traits common to the *L. elegans* complex, but also their basal scar of nuts concave. On the other hand, *L. blaoensis* is distinct from *L. grandifolius* in having shorter infructescences (9–14 cm long vs. 15–20 cm long), cupules enclosing not less than1/2 of the nut (vs. enclosing 1/7–1/6 of nuts), scales on cupule not united into concentric rings (vs. connate into 5–7 horizontal ridges), and smaller basal scar.

Lithocarpus syncarpus (T4709 and V3188 with fruits) is similar to *L. grandifolius* (MY1552) in their largely connate cupules, leaf blade glabrous on both surfaces, cupules enclosing less than 1/4 of the nut, nut depressed ovoid at both ends, and basal scar of nuts concave, but *L. syncarpus* is distinguished from *L. grandifolius* in having more or less shorter infructescences (10–16 cm long in *L. syncarpus* vs. 15–20 cm long in *L. grandifolius*), scales on cupules not formed into concentric rings (vs. connate into 5–7 horizontal ridges), bigger basal scar (12–15 mm in diam. vs. 8–12 mm in diam.).

Two new species, *L. bokorensis* and *L. monoromensis* are morphologically similar in having petioles less than 1.5 cm long, cupules enclosing less than 1/3 of the nut, imbricate scales on cupules, and basal scar concave, but these two species differ in number of secondary veins (8–10 pairs in *L. bokorensis* vs. 11–13 pairs in *L. monoromensis*), length of infructescences (7–8.5 cm long vs. 19–26 cm long), nut size

(10–12 mm tall × 13–14 mm in diam. vs. 15–17 mm× 14–18 mm), cupules enclosing (1/5–1/3 of the nuts vs. 1/7 of the nuts), basal scar size (ca. 8 mm in diam vs. 11–14 mm).

Lithocarpus harmandii subsp. *malacotrichus* is most similar to *L. harmandii* subsp. *harmandii* in having densely tomenstose twigs, same number of secondary veins, long petioles and infructescences but distinguished in its broad elliptic leaves, base obtuse (vs. Ovate-lanceolate, base attenuate in *L. harmandii* subsp. *harmandii*), leaf blade densely white tomentose both surfaces (vs. yellow pubescent on lower surface in *L. harmandii* subsp. *harmandii*), elongate turbinate nuts (vs. obvoid or sub obovoid), basal scar flat or sometimes convex at center (vs. concave).

While the MIG-seq tree and population genetic analysis supported that our samples of the *L. hancei* complex include three species, morphological observations on our collections and herbarium specimens including type specimens revealed that *L. yersinii* may be distinguishable in leaf and fruit morphology but it is very difficult to distinguish *L. hancei* and *L. jacsonianus* by morphology. We identified our collections as *L. hancei, L. jacsonianus* and *L. yersinii* mainly considering geographical distribution; the northernmost clade (V4924, V4800 and V5833) as *L. hancei* described from China, the clade including V3092 collected in the vicinity of the type locality of *L. jacsonianus* as *L. jacsonianus*, and the southernmost clade including three samples collected in the type locality of *L. yersinii* (Mt. Honba) as *L. yersinii.* In addition, we designated V65 and V5111 as aff. *L. yersinii* and aff. *L. hancei*, respectively. We collected both *L. jacsonianus* (V4918, flowering) and *L. hancei* (V4924 and V4800, sterile) in a plot placed from 1885 to 1910 m in Mt. Fansipan, but we could not distinguish *L. jacsonianus* and *L. hancei* there and recorded them as the same species in our plot survey. We also collected aff. *L. hancei* (V5111, flowering) in another plot placed at 2225 m in Mt. Fansipan. In VNM,

there is a fruiting specimen collected from Sapa (a city near Fansipan) at 1800 m (Petelot 8001) that is identified as *L. hancei* or aff. *L. hancei*. This specimen has short infructescences (less than 5 cm) as in herbarium specimens of *L. hancei* collected in China. We collected a fruiting specimen of *L. hancei* (V5833) in Vu Quang National Park, having short infructescences (less than 8 cm). On the other hand, fruiting specimens of *L. jacsonianus* (V7432) collected in Ba Vi National Park of northern Vietnam had longer infructescences (8–11 cm long vs. 6– 8 cm long in *L. hancei*) having much crowded acorns (sparsely arranged in *L. hancei*). The type of *L. jacsonianus* collected from Quang Nam of central Vietnam is a flowering specimen with an apically broken, old infructescence. Acorns are not crowded on this infructescence.

Lithocarpus yersinii was described from Hon Ba Nature Reserve, Khanh Hoa province, where we collected three specimens V106 (sterile), V1424 (flowering), and V2129 (with the first year infructencence in which nuts are not yet developed). In leaves, those collections are different from the collections of *L. hancei* and *L. jacksonianus* in that tertiary veins are indistinct on the lower surface. Observations on the type specimen and *Chevalier A.J.B 38904* showed that acorns are solitary (vs. usually clustered in *L. hancei* and *L. jacksonianus*) and cupule has 3–4 concentric rings (vs. imbricate).

The type specimens of L. sabulicolus is distinct from L. hancei and L. jacksonianus in its shorter petioles (0.5–1 cm long in L. sabulicolus vs. 1.5–4 cm long in L. hancei and 1.2–1.5 cm long in L. jacksonianus), longer infructescences (12–14.5 cm long in L. sabulicolus vs. 6–8 cm long in L. hancei and 8–11 cm long in L. jacksonianus), solitary cupules (vs. clustered of 3–5 in both L. hancei and L. jacksonianus). Lithocarpus sabulicolus is similar to L. yersinii in having same number of secondary veins and solitary cupules but distinguished by its shorter petioles (0.5–1 cm long in L. sabulicolus vs. 1.2–

1.5 cm in L. yersinii), longer infructescences (12–14.5 cm long vs. 8–10 cm long), scales on cupules not united into concentric rings (vs. united into 3–4 concentric rings in L. yersinii).

Population genetic analysis

A population genetic analysis using STRUCTURE revealed that *L. syncarpus* is differentiated to two genetically isolated populations (Fig. 5.3), one in Loei Province, Thailand, and another in Lam Dong Province, Vietnam. In *L. monoromensis*, Delta K was the highest at K = 4 (Fig. 5.4). The bar plot showed that 7277, V5399 and V3167 had mostly unique genetic compositions and 7317 and 7617 are dominated by genotypes from the fourth source population whereas 6903 had a mixture of heterogeneous genetic compositions derived from three source populations. For the *L. hancei* complex, Delta K was the highest at K=3 (Fig. 5.5). The 15 samples of 19 total had homogeneous genetic compositions with 88 % (for V2129) or more genotypes derived from either of three source population, corresponding to *L. jacksonianus* (V6062, V6051, V5441, V5345, V3092, V6043 and V4918), *L. yersinii* (V106, V1424, V4407, V2991, and V2129) and *L. hancei* (V4800 and V4924). Aff. *L. yersinii* (V65) had a homogeneous genetic composition identical with five samples of *L. yersinii.* On the other hand, V5833 (*L. hancei*), V6261 (*L. jacksonianus*), V6400 (*L. jacksonianus*), and V5111 (aff. *L. hancei*) had more heterogeneous genetic compositions with approximately 1:1 mixtures of genotypes derived from two source populations.

Discussion

Taxonomy of the *Lithocarpus elegans* complex

The results from molecular studies and morphological observations showed that the plants treated as *L. elegans* in Vietnam (Ho, 2003), Cambodia (Tagane et al. 2017), Thailand (Phengklai, 2008) and Myanmar (Kress et al. 2003) are not a single widespread species but include five different species: *L. blaoensis, L. bokorensis, L. grandifolius, L. monoromensis* and *L. syncarpus*.

Among five species of the *L. elegans* complex, *L. blaoensis* was clustered not with the other species of the *L. elegans* complex but with *L. petelotii* and a clade consisted of *L. blaoensis* and *L. petelotii* was sister to the *L. hancei* complex, not to the *L. elegans* complex. Although *L. blaoensis* was treated as a synonym of *L. elegans* by Ho (2003), the above evidence supports that *L. blaoensis* is a distinct species not so close to the *L. elegans* complex. The range of *L. blaoensis* is remotely isolated from that of *L. grandifolius*; while *L. grandifolius* is widely distributed in Nepal, Myanmar, northern Thailand and southern China, *L. blaoensis* has been known to be restricted to a small area in Lam Dong Province of southern Vietnam. The range of *L. blaoensis* may be broader because the sterile samples collected from Bach Ma National Park of central Vietnam (V2995 and V3005, identified as *L.* aff. *blaoensis*) were clustered with *L. blaoensis* (V3176, a collection from the type locality, having fruits).

Lithocarpus grandifolius was placed in the basal position of the *L. elegans* complex. Among the other species, *L. bokorensis* was sister to a pair of species, *L. harmandii* subsp. *harmandii* and *L. harmandii* subsp. *malacotrichus*. This finding is unexpected because the two species are not similar to any species of the *L. elegans* complex in having densely hairy leaves. By this diagnostic trait, *L. harmandii* subsp. *harmandii* is widely accepted and has never been reduced to *L. elegans s. lat.* (Hickel & Camus 1921; Ho, 2003; Phengklai, 2008). *Lithocarpus harmandii* subsp. *malacotrichus*

was sister to *L. harmandii* subsp. *harmandii* and distinguished from this species as a subspecies, considering that it is distributed from central Laos (*Poilane E. 15927*, *Yahara L1818, L1835* & *L1841*) to southern Vietnam (*Ngoc V3171*) and geographically isolated from *L. harmandii s. str*.

A clade including *L. bokorensis, L. harmandii* subsp. *harmandii* and *L. harmandii* subsp. *malacotrichus* is sister to *L. monoromensis*, and the clade including those four species is sister to *L. syncarpus*. Thus, by recognizing *L. harmandii* subsp. *harmandii*, a widely accepted species (Hickel & Camus 1921; Ho, 2003; Phengklai, 2008), we need to distinguish *L. bokorensis, L. monoromensis* and *L. syncarpus* as three different species to avoid naming a paraphyletic group as a species. Morphological observations also supported that *L. bokorensis, L. monoromensis* and *L. syncarpus* are distinguishable by diagnostic traits described above. Although, two samples collected from Thailand and three samples collected from Vietnam were differentiated to two genetically isolated populations corresponding to their geographical distributions, and the specimens from Phu Kradueng, Thailand, has smaller and narrower leaves, and less prominent tertiary veins than those of Vietnam, but the other characters including fruits are highly similar each other and indistinguishable. Thus, we identified those five specimens as a single species, *L. syncarpus*. While *L. bokorensis* is endemic to Mt. Bokor, southwestern Cambodia and *L. monoromensis* is restricted to a narrow area from Mondulukiri, western Cambodia to Dong Nai and Lam Dong, southern Vietnam, *L. syncarpus* is more widely distributed from Vietnam to northeastern Thailand. Both *L. monoromensis* and *L. syncarpus* are collected in Lamdong Province, Vietnam where we did not find any intermediate, supporting that these two are different species.

The population genetic analysis showed *L. monoromensis* is genetically heterogeneous, including genetic compositions derived from four source populations. This evidence suggests that *L. monoromensis* was once differentiated to plural genetically isolated populations, but those populations are now hybridizing. Further molecular studies using more individuals are needed to elucidate population genetic structure and evolutionary history of *L. monoromensis*.

We did not include *L. elegans s. str.* in the MIG-seq analysis, but morphological observations on our collections of *L. blaoensis, L. bokorensis, L. grandifolius, L. monoromensis* and *L. syncarpus* and the specimens of *L. elegans* collected in Java including the type specimens showed that *L. elegans* is identical with neither of the five species; glabrous and greenish leaf blade, cuneate or acute base, cupules deeply cuneate when mature, cupule scales imbricate (somtimes more or less concentrically set), nuts glabrous, basal scar concave. Cockburn (1972) and Soepadmo (1972) treated *L. elegans* as a morphologically variable and widely distributed species in Malesiana region However, our results suggest that *L. elegans* in the sense of Cockburn (1972) and Soepadmo (1972) may include some cryptic species. Further studies are needed to revise taxonomy of *L. elegans* in the Malesiana region.

Taxonomy of the *Lithocarpus hancei* complex

In the MIG-seq tree of the *L. hancei* complex (Fig.5.2), V65 and V5111 identified as *L. yersinii* and *L. hancei*, respectively, not placed on the same clades with the rest five samples of *L. yersinii* on clade 2B2 and the rest three samples of *L. hancei* on clade 2B3, but clustered with nine samples of *L. jacsonianus* in the clade h1. Population genetic analysis with STRUCTURE showed that the samples of the *L. hancei* complex include three different source populations, roughly corresponding to *L.*

jacksonianus, L. hancei and *L. yersinii*. Seven samples of *L. jacksonianus* but excluding V6261 and V6400 that were clustered within the clade of the rest nine samples of *L. jacsonianus* showed a uniform composition derived from single source population. V6261 and V6400 showed a nearly 1:1 mixture of genetic compositions derived from two source populations. V5111 and V5833 identified as *L. hancei* also had a heterogeneous profile with a nearly 1:1 mixture of genetic compositions derived from two source populations; V5111 had a lower proportion of another element from the third source population. The other two samples of *L. hancei*, V4800 and V4924, had a uniform composition derived from single source population. It is notable that V4800 and V4924 identified as *L. hancei* were collected in the same stand at ca. 1900 m of Mt. Fan Si Pan where V4918 of *L. jacksonianus* was also collected. The differentiation between those samples of *L. hancei* and *L. jacksonianus* in Fan Si Pan shown in both the MIG-seq tree and STRUCTURE profile support that *L. hancei* and *L. jacksonianus* are two reproductively isolated species at least in Fan Si Pan. Two other samples identified as *L. hancei* (V5111 collected at 2225 m of Fan Si Pan and V5833 collected at 1726 m of Vu Quang) having the mixed population genetic profile might be of hybrid origin between *L. hancei* and another species. Although, V5111 did not nested with three other samples of *L. hancei* in the MIG-seq tree, but based on morphological evidence and population genetic analysis we identified that sample as *L.* aff. *hancei,* we need further studies to clarify the identity of this sample. Because *L. hancei* is distributed wdely in China and highly variable there, further studies on Chinese populations are needed to elucidate evolutionary history and phylogenetic divergence of this species.

Three samples of *L. yersinii* (V106, V1424 and V2129) and one sample idenfitied as *L.* aff. *yersinii* (V65) were collected at 1498 m to 1545 m of the type locality of *L. yersinii*, Hon Ba Nature Reserve, southern Vietnam. Among them, V65 was

separated from the five remaining samples of *L. yersinii* in the MIG-seq tree (Fig. 2), but in the STRUCTURE profile (Fig. 6) V65 and two other samples (V106, V1424) collected from Hon Ba had a uniform composition derived from single source population. Two other samples identified as *L. yersinii* (V4407 collected at 1544 m of Bidoup-Nui Ba neighboring to the type locality of *L. yersinii,* and V2991 collected at 1412 m from Mt. Bach Ma) were clustered with three other samples collected from Hon Ba (excluding V65) in the same clade of the MIG-seq tree, and also showed the uniform STRUCTURE profile derived from single source population. The congruence in the MIG-seq tree and STRUCTURE profile provided evidence that *L. yersinii* is a genetically distinct species, although further studies are needed to determine the identity of *L.* aff. *yersinii* (V65).

Key to the species of *L. elegans* and its relatives

1. Petioles less than 1.5 cm long; cupules enclosing less than 1/3 of the nut.
   2. Infructescences longer than 14 cm long, basal scar of nut 10–15 mm in diam.
      3. Leaf blade narrowly to broadly obovate or elliptic; cupule enclosing 1/4–1/2 of the nut, scales on cupules more or less united into concentric rings ..... ***L. elegans***
      3. Leaf blade narrowly oblong-elliptic to oblanceolate; cupules enclosing 1/7 of the nut, scales on cupules always imbricate ..... ***L. monoromensis***
   2. Infructescences less than 10 cm long, basal scar less than 10 mm in diam ..... ***L. bokorensis***
1. Petioles 1.5–4 cm long; cupules enclosing more than 1/3 of the nut.
   4. Scales on cupules connate into 5–7 concentric rings ..... ***L. grandifolius***
   4. Scales on cupules imbricate
      5. Leaf blade ovate, ratio of petiole/blade length 0.15–0.18; nut 17–19 mm tall × 17–18 mm in diam. ..... ***L. blaoensis***
      5. Leaf blade ovate-lanceolate, ratio of petiole/blade length 0.13–0.15; nut 12–13 mm tall × 15–17 mm in diam. ..... ***L. syncarpus***

Key to the species of *L. hancei* and its relatives

1. Cupule clustered of 3 or more .....***L. hancei*** and ***L. jacksonianus***
1. Cupules solitary, never clustered.
   3. Infructescences 12–14 cm long; cupule enclosing 1/4 of the nut, scales imbricate. .....***L. sabulicolus***
   3. Infructescences 8–10 cm long; cupules enclosing 1/3 of the nut, scales united into 3–4 concentric rings. ..... ***L. yersinii***

Taxonomic treatments

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Type. VIETNAM. Lam Dong Province "Annam: Station agricole de Blao, prov. du Haut Donaï" 800 m alt., 25 Jun. 1933, *Poilane E. 22750* [fr.] (lectotype: P [P00744443, image!]; isolectotypes: P [P00744444, image!], VNM [VNM00020005!], **designated here**).

Additional specimens examined. VIETNAM. Lam Dong Province: "Annam: Station agricole de Blao, prov. du Haut Donaï", 500 m alt., 7 Sep. 1930, *Poilane E. 18272* [fr.] (P [P00744447, image!]); ibid., 800 m alt., 27 Mar. 1933, *Poilane E. 22238* [fr.] (P [P00744445, image!]); ibid., 24 Apr. 1933, *Poilane E. 22391* [fr.] (P [P00744446, image!]); ibid., 20 Dec. 1933, *Poilane E. 21984* (VNM [VNM00020006!]); ibid., 11°43'37.0"N, 107°42'34.5"E, 1000 m alt., 13 Jun. 2015, *Nguyen N., Luong D., Hoang B., Nguyen T. V3176* [fr.] (DLU!, FU!). Thua Thien Hue Province: Bach Ma National

Park, in evergreen forest, 16°11' 56.87"N, 107°51' 26.01"E, 1412 m alt., 27 May 2015, *Yahara T., Tagane S., Toyama H., Nguyen N., Nguyen C., Okabe N. V2995* [ster.] (DLU!, FU!); ibid., 16°11' 49.05"N, 107°51' 22.43"E, 1348 m alt., 27 May 2015, *Yahara T., Tagane S., Toyama H., Nguyen N., Nguyen C., Okabe N. V3004* [fr.] (DLU!, FU!). Kon Tum Province: Ngoc Linh Nature Reserve, in hill evergreen forest, 15°10'07.4"N, 107°45'43.4"E, 1376 m alt., 13 Feb. 2017, *Tagane S., Nagamasu H., Nguyen Van Ngoc, Hoang Thi Binh, Hoang Thanh Son, Yang C.-J., Kawakubo A. V6524* [ster.] (DLU!, FU!); ibid., 15°12'24.2"N, 107°46'10.4"E, 1365 m alt., 14 Feb. 2017, *Tagane S., Nagamasu H., Nguyen Van Ngoc, Hoang Thi Binh, Hoang Thanh Son, Yang C.-J., Kawakubo A. V6634* [ster.] (DLU!, FU!). Da Nang City, Ba Na, Mt. Ba Na, 15°59'51"N, 107°59'17"E, 1450 m alt., 19 Feb. 2017, *Tagane S., Nagamasu H., Nguyen Van Ngoc, Hoang Thi Binh, Hoang Thanh Son, Kawakubo A. V6956* [ster.] (DLU!, FU!).

Distribution. Vietnam: (Lam Dong Province - type locality, Kon Tum, Thua Thien Hue).

Lithocarpus bokorensis Ngoc, Tagane & Yahara, **sp. nov.**

Lithocarpus elegans auct. non (Blume) Hatus. ex Soepadomo; Tagane et al. A Picture Guide of Tree Flora of Cambodia IV: 267 (2017).

Fig. 5.6

Type. CAMBODIA. Kampot Province, Mt. Bokor, in the evergreen forest, 10°38'12.59"N, 104°02'06.37"E, 1014 m alt., 20 Jul. 2012, *Tagane S., Fuse K., Chhang P. 4131* [male fl., fr.] (holotype: KYO!; isotypes: FU!, P!).

Diagnosis. *Lithocarpus bokorensis* is distinct from *L. elegans* in having smaller leaves with fewer secondary veins, shorter infructescences and smaller nuts. In Cambodia, *L. bokorensis* is most similar to *L. monoromensis* but differs in having longer petioles (1–2

cm long vs. 0.5–0.9 cm in *L. monoromensis*), shorter infructescences (7–8.5 cm long vs. 19–26 cm long), smaller nuts (12–14 mm tall × 13–16 mm in diam. vs. 15–17 mm tall × 14–18 mm in diam.) and smaller basal scar of nut (9–11 mm in diam. vs. 11–14 mm in diam.), (Table 5.1).

Description. Trees, ca. 8 m tall. Terminal bud ellipsoidal-ovoid, ca. 4 × 2.5 mm, bract ovate to ovate-triangular, ca. 2 mm long, apex acute, pubescent to glabrescent outside, glabrous inside. Young branches short hairy, soon glabrous, yellowish green *in vivo*, brownish *in sicco*, old branches grayish brown, lenticellate. Stipule narrowly triangular, 2–2.5 × ca. 1.1 mm long, margin ciliate. Leaves alternate, spirally arranged; blade obovate, oblong-elliptic, elliptic, 3.7–13 × 2.1—4.8 cm, coriaceous, glabrous on both sides, apex attenuate to acuminate, acumen up to 1 cm long, base cuneate to attenuate, margin entire, recurved when dry, midrib slightly prominent near base adaxially, prominent abaxially, secondary veins 8–10 pairs, at an angle of ca. 50 degree from the midrib, prominent abaxially, tertiary veins scalariforming-reticulate, faintly visible on both sides; petiole 0.2—1.1 cm long, glabrous. Male inflorescence terminal or axial near the shoot apex, a spike, –2.8–7.5 cm long, tomentose. Male flower in 3 flowered clusters, bract narrowly triangular, ca. 1.2 mm long, sparsely appressed hairy, calyx 6-lobed, lobes ovate, 0.75–1 mm × 0.5 mm, densely white tomentose outside, glabrous inside; stamens 12, 1.25–1.5 mm long, anthers 0.25 mm long. Fruiting spike erect, woody, 7–8.5 cm long, axis ca. 4 mm thick at base, grayish brown, (sparsely) tomentose, lenticellate. Cupule clustered of 3, sessile, broadly saucer-shaped, 0.5 cm tall, 1.2–1.5 cm in diam., enclosing basal 1/5–1/3 of the nuts, glabrous; scales on cupule triangular, imbricate, apex shortly acuminate, tomentose; Nut broadly ovoid, depressed at both ends, 1–1.2 cm tall, 1.3–1.4 cm in diam., glabrous; basal scar concave, ca. 0.8 cm in diam., glabrous.

Distribution. Cambodia (Kampot Province-type locality)

Habitat and ecology. *Lithocarpus bokorensis* is scattered in moist evergreen forest on the top plateau of Mt. Bokor, at alt. 1000 m. Flowering and fruiting specimens were collected in July.

Etymology. The species epithet is derived from its type locality, Mt. Bokor, Kampot Province, Cambodia.

Note. The sterile specimens from Koh Kong, Cambodia (11°32'10.21"N, 103°09'46.85"E, 183 m alt., 7 Dec. 2012, *Toyama et al. 4713*, FU) is phylogenetically a sister to *L. bokorensis*, but it possesses non-lenticellate twigs, much larger leaves (9–20 × 3.4–8.2 cm) and more secondary veins (ca. 16 pairs vs. 8–10 pairs in *L. bokorensis*). We need additional fruiting materials for examine its taxonomic position.

Lithocarpus elegans (Blume) Hatus. ex Soepadomo, Reinwardtia 8: 236 (1970); Fl. Males. ser.I, 7: 366 (1972), p.p.; Phengklai, Fl. Thai. 9: 279, nom. tant.; Ho, Ill. Fl. Viet. 2: 635, nom. tant.

Quercus elegans Blume, Verh. Batav. Genootsch. Kunsten 9: 208 (1825).

Type. INDONESIA. Java, *Blume C.L. von. s.n.* [fr.] (holotype: L; isotypes: K [K000832510, K000832511, K000832512, images!], P [P00744450, image!]).

Additional specimens examined. INDONESIA. Java, *Blume C.L. von. s.n.* [fr.] (HBG [HBG-516785, image!]); ibid., *s.coll. s.n.* [fr.] (K [K000832509, image!]); Gede Pangorango National Park, along the trail from Cibodas to Kandang Badak, 06°45' 54.94"S, 106°58'58.83"E, 2120 m alt., 2 Oct. 2011, *Yahara T., Nagamasu H., Naiki A., Toyama H., Ichihashi R., Hidayat A., Sadili A., Ardiyani M., Darnaedi D. IJ796* [ster.], *IJ822* (FU!); ibid., 06°46'22.12"S, 106°58'35.84"E, 2310 m alt., 5 Oct. 2011, *Yahara T.,*

Nagamasu H., Naiki A., Toyama H., Ichihashi R., Hidayat A., Sadili A., Ardiyani M., Darnaedi D. IJ1047 [ster.] (FU!); ibid., 5 Oct. 2011, *Yahara T., Nagamasu H., Naiki A., Toyama H., Ichihashi R., Hidayat A., Sadili A., Ardiyani M., Darnaedi D. IJ1047* [ster.] (FU!); ibid., 06°46.557'S, 107°58.607'E, 2400 m alt., 8 Oct. 2011, *Tagane S., Jaeni A. IJ1108* [ster.] (FU!). Sumatra, Bangka, *Teijsmann J.E. 7640* [male fl.] (K [K000832508, image!]); ibid., *Teijsmann J.E. 7641* [ster.] (L [L0040679, image!], K [K000832508, image!]). MALAYSIA. Sabah, Kituntul, Ranau, 6 Jan. 1988, *Amin et al. SAN123588* [fr.] (L [L.3800514, image!]). Kelantan, Dabong, 5°25'0"N, 102°0'0"E, 20 Aug. 1990, *Soepadmo E., Suhaimi M., S300* [fr.] (L [L.3789533, image!]). Pahang, Raub, Fraser's Hill, Richmond, Kuala Kubu Bharu-Fraser Hills Rd., 14 Mar. 2012, *Imin K., Phoon S.N., Mohd Nazri A., FRI 76232* [fr.] (L [L.3793568, L.3793569, image!]).

Distribution. Indonesia (Java - type locality), Malaysia.

Lithocarpus grandifolius (D.Don), Biswas., Bull. Bot. Surv. India 10: 258 (1969); Huang et al., Fl. China 4: 364 (1999).

Quercus grandifolia D. Don, in Lamb. Gen. Pin. 2: 27 (1824).

Lithocarpus spicatus (Sm.) Rehder & E.H.Wilson, Pl. Wilson. (Sargent) 3(2): 207 (1916), nom. illegit.

Quercus spicata Sm., Cycl. [A. Rees], (London ed.) 29: 12 (1819), nom. illegit.

*Lithocarpus elegan*s auct. non (Blume) Hatus. ex Soepadomo; Phengklai, Fl. Thailand 9: 279 (2008), p.p.

Type. NEPAL. "Sillet." *Herb Wallich N., s.n*. [fl.] (lectotype: L [L1554601, image!], **designate here**)

Additional specimens examined. CHINA. Sichuan Province, Emeishan, 1600 m alt., Oct. 1957, 植被组 *3235* [fr.] (CDBI [CDBI0014225, image!]). Yunnan Province,

Xishuangbanna, Menghai, Nannuo Shan to Menghai, Nov. 1955, 毛品一 *7319* [fr.] (IBSC [0037770, 0037773, image!]); ibid., Mar. 1957, 中苏队 *2* [ster.] (IBSC [IBSC0037781, image!]); ibid., Shuangjiang, Kenshin Zhang, 1500 m alt., Sep. 1957, 辛景三 *978* [fr.] (IBSC, [IBSC0037776, image!]); ibid., Mengla, 600 m alt., 12 Oct. 1959, 蔡希陶 *59-10926* [fr.] (NAS [NAS00202633, image!]); ibid., Simao, 1200 m alt., Apr. 1959, 中苏队 *8175* [male fl.] (IBSC [IBSC0037777, image!]); ibid., Ruili, 7 Apr. 1961, 周铉 *523* [male fl.] (HITBC [HITBC021989, image!]). INDIA. Khasia hills, *Hooker J.D., Thomson T., s.n.* [fl.] (L [L1554542, L1554543, image!]); ibid., *Masters s.n.* [fl.] (L [L1554545, L1554546, image!]); ibid., 5 Apr. 1894, *Gammie G.A. 401* [fl.] (L [L1554544, image!]). MYANMAR. Kachin Province, Indawgyi Wildlife Sanctuary, Mohnyin Township, Kachin State, in hill evergreen forest, 24°58'03.84"N, 96°22'32.88"E, 839 m alt., 11 Dec. 2016, *Tagane S., Nagamasu H., Okabe N., Mu Mu Aung, Yunn Mi Mi Kyaw, Awng Khine Win MY1552* [fr.] (FU!, RAF, TNS). THAILAND. Doi Inthnanon National Park, Chiang Mai, 700 m alt., *Wongprasert T.H., Khaoiam S. 038-123* [fr.] (BKF!).

Distribution. Bhutan, China (S to SW Yunnan), India, Myanmar, Nepal (type locality), Thailand (northern).

Lithocarpus hancei (Benth.) Rehder, J. Arnold Arbor. 1: 127 (1919); Ho, Ill. Fl. Viet. 2: 638, Fig. 6546 (2003).

Quercus hancei Benth., Fl. Hongk.: 322 (1861).

Type. CHINA, Hong Kong, in a ravine of Mt. Gough, Feb. 1858, *Wilford C. s.n.* [male fl., fr.] (lectotype: BM [BM000951906, image!]; isolectotypes: GH [GH00033899, image!], K [K000297006, image!], **designate here**).

Additional specimens examined. CHINA. Hong Kong: 1853, *Wright C. 463* [male fl.] (K [K000832359, image!]); ibid., Victoria Peak, 1854, *Champion 494* [fl.] (K [K000297005, image!]); ibid., Nov. 1862, *Hance H.F. 1525* [fl., fr.] (K, [K000832360, K000297004, image!]). VIETNAM. Lao Cai Province: Sapa, 1942, *Petelot 8001* [fr.] (VNM [VNM00020277!, VNM00020277!]); ibid., Hoang Lien National Park, Mt. Fansipan (Phang Xi Pang), in lower montane evergreen forest, 22°20'57.1"N, 103°46'15.4"E, 1900 m alt., 30 Apr. 2016, *Nguyen V.N., Hoang T.B., Yahara T., Toyama H., Tagane S., Nagamasu H., Naiki A., Hoang T.S. V4800* [ster.] (DLU!, FU!); ibid., 1 May 2016, *Nguyen V.N., Hoang T.B., Yahara T., Toyama H., Tagane S., Nagamasu H., Naiki A., Hoang T.S. V4924* [ster.] (DLU!, FU!); ibid., 22°19'48.0"N, 103°46'57.5"E, 2225 m alt., 01 May 2016, *Nguyen V.N., Hoang T.B., Yahara T., Toyama H., Tagane S., Nagamasu H., Naiki A., Hoang T.S. V5111* [fl.] (DLU!, FU!). Ha Tinh Province: Vu Quang National Park, in lower montane forest, along the trail to the summit of Rao Co, 18°11'41.7"N, 105°23'06.1"E, 1726 m alt., 23 Jun. 2016, *Yahara T., Nguyen V.N., Toyama H., Tagane S., Okabe N., Nguyen V.H. V5833* [young fr.] (DLU, FU!).

Distribution. China (Hong Kong - type locality), Vietnam.

Lithocarpus harmandii (Hickel & A.Camus) A.Camus, Rivièra Sci. 18: 40 (1931); Ho, Ill. Fl. Viet. 2: 638, Fig. 6547 (2003).

Pasania harmandii Hickel & A.Camus, Ann. Sci. Nat., Bot. 10(3): 390 (1921).

Type. CAMBODIA. "Bassin du Sè-Moun: Vallée de Compong Xoai", Feb. 1876, *Harmand F.J. 400.* [female fl., fr.] (Lectotype: P [P00744410, image!]; isolectotypes: BM [BM000951930, BM000951931, image!], P [P00744411, P00744413, image!], **designated here**).

Additional specimens examined. CAMBODIA. Kampot Province: “Nord de Kampot” 04 Feb. 1928, *Poilane E. 14652* [fl.] (P [P00544063, image!]). Koh Kong Province: Kirirom, Mar./Jun. 1967–1968, *Dy Phon M. 1183* [fr.] (P [P00544060, P00544061, image!]). Kampong Thom Province, regrowth forest, 12°22'48.54"N, 105°9' 43.59"E, 32 m alt., 22 Nov. 2010, *Toyama H., Kajisa T., Ichihashi R., Itadani H., Katayama A., Tachiki Y., Chhang P., Vanna S., Vineth S., Yahara T. 379* [fr.] (FU!, the Forest Administration of Cambodia); ibid., 12°22'48.47"N, 105°9'40.93" E, 32 m alt., 22 Nov. 2010, *Toyama H., Kajisa T., Ichihashi R., Itadani H., Katayama A., Tachiki Y., Chhang P., Vanna S., Vineth S., Yahara T. 419* [fr.] (FU!, the Forest Administration of Cambodia); ibid., 12°22'48.75"N, 105°9'17.32"E, 26 m alt., 25 Apr. 2011, *Toyama H., Tagane S., Kajisa T., Onoda Y., Chhang P., Syneath S., Yahara T. 1023* [ster.] (FU!, the Forest Administration of Cambodia). VIETNAM. Tay Ninh Province: “Cochinchine: Ti Tinh” 1862–1866, *C. Thorel s.n.* [fr.] (P [P00744412, image!); ibid., “Ad Cai Cong” Apr. 1866, *L. Pierre 4966* [fr.] (G [G003358041, G00358042, image!], K [K000832474, image!], P [P0074441, image!]). Ba Ria Vung Tau Province: “Ad Noc in prov. Baria austrocochinchinae” Dec. 1865, *L. Pierre 4966* [fr.] (P [P00744416, image!]). Binh Duong Province: “In prov. Thu Dzau Mot ad Ben Cât austro Cochinchinae” Dec. 1866, *Pierre L. 4966* [fr.] (P [P00744415, P01044192, P01044193, image!]).

Distribution. Cambodia (Kampot - type locality), Vietnam.

Lithocarpus harmandii* (A.Camus) subsp. *malacotrichus A.Camus, Bull. Soc. Bot. France 94: 270 (1948).

Type. LAOS. “Province d'Attopeu: Entre B. Thuot et Phu Da Phuk, plateau des Bolovens”, 650 m alt. 8 Oct. 1928, *Poilane E. 15927* [fr.] (holotype: P [P00744488, image!]; isotype: P [P00744489, image!]).

Additional specimens examined. LAOS.Vientiane Province: Thoulakhom District, Ban Pa Paek, Phou Khao Khouay National Protected Area, in open pine forest, 18 22'35.28"N, 102 51'29.46"E, alt. 905 m. *Yahara T., Tagane S., Souladeth P., Nagamasu H., Naiki A., Chayer S. & Dueantaa E. L1818* [fr.], *L1835* [fr.], *L1841* [ster.] (FOF, FU!). VIETNAM. Lam Dong Province, Duc Trong, Lang Hanh, edge of evergreen forest, 11°37'44.3"N, 108°16'30.1" E, 890 m alt., 13 Jun. 2015, *Nguyen N., Luong D., Hoang B., Nguyen T. V3171* [young fr.] (DLU!, FU!).

Distribution. Laos (Attopeu - typelocality, Vientiane), Vietnam (Lam Dong).

Lithocarpus jacksonianus A.Camus, Bull. Soc. Bot. France 92: 83 (1946); Ho, Ill. Fl. Viet. 2: 639, Fig. 6549 (2003).

Type. VIETNAM. Quang Nam Province, "Annam: Province Quang Nam, poste 6", 700 m alt., 21 Mar. 1939, *Poilane E. 29503* [fl.] (holotype: P [P00744465!]; isotype: US [US00089370, image!]).

Additional specimens examined. VIETNAM. Lao Cai Province: Fansipan, Hoang Lien National Park, Mt. Fansipan (Phang Xi Pang), in lower montane evergreen forest, 22°21'04.3"N, 103°46'20.7"E, 1919 m alt., 1 May 2016, *Nguyen V.N., Hoang T.B., Yahara T., Toyama H., Tagane S., Nagamasu H., Naiki A., Hoang T.S. V4918* [fl.] (DLU!, FU!); ibid., 22°19'47.5"N, 103°49'18.0"E, 5 May 2016, *Nguyen V.N., Hoang T.B., Yahara T., Toyama H., Tagane S., Nagamasu H., Naiki A., Hoang T.S. V5345* [fl.] (DLU!, FU!). Nghe An Province: Pu Mat National Park, Kem Waterfall, along the trail in the montane evergreen forest, 19°00'20.3"N, 104°48'10.9"E, 400 m alt., 15 May 2016, *Nguyen V.N., Hoang T.B. V5441* [ster.] (DLU!, FU!). Ha Noi Capital: Ba Vi National Park, 21°03'43.4"N, 105°21'39.7"E, 1125 m alt., 16 Sep. 2016, *Bon V6045* [ster.], *V6051* [ster.],

V6062 [ster.] (FU!); ibid., 21°03.622"N, 105°21.747"E, 22 Sep. 2017, *Yahara T., Ngoc N.V., Binh H.T., Mase K., Son H.T. V7432* [state the condition]*, V7433* [fr.] (DLU!, FU!). Da Nang, Ba Na Nature Reserve, edge of evergreen forest, 16°00'07.30"N, 108°01' 33.90" E, 707 m alt., 29 May 2015, *Tagane S., Toyama H., Nguyen N., Nguyen C. V3092* [ster.] (DLU!, FU!). Kon Tum Province, Ngoc Linh Nature Reserve, in hill evergreen forest, 15°10'05.7"N, 107°45'23.6"E, 1067 m alt., 11 Feb. 2017, *Tagane S., Nagamasu H., Ngoc N.V., Binh H.T., Son H.T., Yang C.-J., Kawakubo A. V6261* [ster.] (DLU!, FU!); ibid., 15°10'5.7"N, 107°45'23.58"E, 1376 m alt., 12 Feb. 2017, *Tagane S., Nagamasu H., Ngoc N.V., Binh H.T., Son H.T., Yang C.-J., Kawakubo A. V6400* [ster.] (DLU!, FU!).

Distribution. Vietnam (Quang Nam - type locality, Lao Cai, Ha Noi, Nghe An, Da Nang, Kon Tum).

Lithocarpus monoromenis Ngoc, Tagane & Yahara, sp. nov.

Fig. 5.7

Diagnosis: *Lithocarpus monoromensis* is phenotypically most similar to *L. bokorensis* but distinguished by its narrowly lanceolate or obalanceolate leaves, shorter petioles, longer infructescences and bigger concave basal scar. *Lithocarpus monoromensis* is also similar to *L. elegans* but distinct by its glabrous twigs (vs. greyish pubescent then glabrescent in *L. elegans s. str.*), leave narrowly lanceolate to oblanceolate, base attenuate (vs. narrowly to broadly obovate or elliptic, base acute, cuneate sometimes rounded or auriculate) scales imbricate (vs. more or less concentrically), nut globose or depressed globose (vs. ovoid or depressed ovoid to sub-globose), basal scar usually concave (vs. sometimes flat) (Table 5.1).

Type. CAMBODIA. Mondulkiri Province, Sen Monorom, roadside to O'romis Hydropower station, edge of evergreen forest 12°29'22.8"N, 107°10'42.2"E, 771 alt., 4

Nov. 2016, *Tagane S., Zhang M., Chhang P., Hatake K., Ota T., Mase K. 7317* [fr.] (holotype: KYO!; isotype: FU!, P!, the Forest Administration of Cambodia).

Description: Trees, ca. 22 m tall. Young branches glabrous, reddish brown, old branches grayish brown, lenticellate. Stipule narrowly triangular, ca. 1.5 mm long, glabrous. Leaves alternate, spirally arranged, blade narrowly oblong-elliptic to oblanceolate, 6.3–15 × 2.2–5.3 cm, thinly coriaceous, greenish yellow *in vivo*, pale greenish brown *in sicco*, glabrous on both sides, apex acuminate, attenuate, base cuneate to attenuate, margin entire, midrib slightly prominent on upper side, prominent on lower side secondary veins 11–13 pairs, at an angle of ca. 55 degree from the midrib, flat on upper surface, slightly prominent on lower surface, tertiary veins scalariforming-reticulate, indistinct adaxially, faintly visible abaxially; petiole 0.5–1.3 cm long, glabrous. Male inflorescences not seen. Female inflorescences erect, woody, 14.5–23 cm long, densely hairy. Infructescences erect, woody, 19–26 cm long, grayish brown, sparsely hairy to glabrous, lenticellate. Cupule clustered of 3 to 7, saucer-shaped, 0.3–0.4 cm tall,1.3–1.5 cm in diam., enclosing ca. 1/7 of the nuts; scales on cupule triangular, imbricate, densely hairy; fruiting stalk almost sessile. Nut depressed ovoid, 1.5–1.7 cm tall, 1.4–1.8 cm in diam., glabrous; basal scar concave, ca. 1.1–1.4 cm in diam.

Additional specimens examined. CAMBODIA. Mondulkiri Province, Sen Monorom, roadside to O'romis Hydropower station, edge of evergreen forest, 12°24'43.32"N, 107°11'04.78"E, 668 m alt., 25 Jan. 2015, *Tagane S., Naiki A., Chhang P. 6903* [female fl. & fr.] (FU!, the herbarim of Forest Administration of Cambodia); ibid., 12°29'22.8"N, 107°10'42.2"E, 771 m alt., 4 Nov. 2016, *Tagane S., Zhang M., Chhang P., Hatake K., Ota T., Mase K. 7277* [fr.] (FU! , the herbarim of Forest Administration of Cambodia); ibid., 12°24'51.4"N, 107°11'18.7"E, 667 m alt., 7 Nov. 2016, *Tagane S., Zhang M.,*

Chhang P., Hatake K., Ota T., Mase K. 7617 [fr.] (FU!, the herbarium of Forest Administration of Cambodia). VIETNAM. Lam Dong Province: Mimosa Pass, 11°54' 59.8"N, 108°27' 27.8"E, 1400 m alt., 13 Jun. 2015, Nguyen N., Luong D., Hoang B., Nguyen T. *V3167* [fl.] (DLU!, FU!). Dong Nai Province, Vinh Cuu, Dong Nai Culture & Nature Reserve, 11°12'23.1"N, 107°04'06.6"E, 89 m alt., 13 May 2016, *Nguyen V.N, Hoang T.B. V5399* [ster.] (DLU!, FU!).

Distribution. Cambodia (Mondulkiri-type locality). Vietnam (Lam Dong, Dong Nai).

Habitat and ecology. *Lithocarpus monoromensis* is not rare in hill evergreen forest along stream. Female inflorescences were collected in January and Fruiting specimens in November.

Etymology. The species epithet is derived from its type locality, Sen Monourom, Mondulkiri Province, Cambodia.

Lithocarpus sabulicolus (Hickel & A.Camus) A.Camus, Riviera Sci. 18: 42 (1931); Ho, Ill. Fl. Viet. 2: 651, Fig. 6597 (2003).

Pasania sabulicola Hickel & A.Camus, Ann. Sci. Nat., Bot., sér. 10, 3: 389 (1921).

Type. Vietnam, Thua Thien Hue Province, "Iter Mékong-Hué: In locis sabulosis prope oppidum Hué", Sep. 1877, *F.J.Harmand s.n.* [fr.] (holotype: P [P00744451, image!]; isotypes: P [P00744452, P00744453, image!]).

Distribuition. Vietnam: (Thua Thien Hue - type locality).

Note. The holotype specimens in P [P00744451] was carefully examined and compared with author's original description and also with isotype specimens ([P00744452, P00744453). We found that the holotype [P00744451] includes two different species. One species is *L. sabulicolus* including several parts of specimens which is

infructescences with solitary cupules, and the remaining parts showing clustered of 3 belong to another species (could be *L. jacksonianus*).

Lithocarpus syncarpus A.Camus, Bull. Soc. Bot. France 85: 654 (1938 publ. 1939); Ho, Ill. Fl. Viet. 2: 652 (2003).

Lithocarpus elegans auct. non (Blume) Hatus. ex Soepadomo; Ho, Ill. Fl. Viet. 2: 635, p.p.

Type. VIETNAM. Dong Nai Province, "Province Bien Hoa: Gia Ray, montagne de Chuia Chan" 800 m alt., 31 Jan. 1921, *Poilane E. 2460* [fr.] (holotype: P [P00744404, image!]; isotype: P [P00744405, P00744406, images!]).

Specimens examined. VIETNAM. Lam Dong Province: Bao Lam, B40 Pass, 11°43'37.0"N, 107°42'34.5"E, 1000 m alt., 13 Jun. 2015, *Nguyen N., Luong D., Hoang B., Nguyen T. V3188* [fr.] (DLU!, FU!); ibid., Di Linh, 11°28'23.5"N, 108°03'58.9"E, 1100 m alt., 21 Jun. 2016, *Nguyen N., Luong D., Hoang B. V3246* [fr.], *V3250* [fr.] (DLU!, FU!). THAILAND. Loei Province: Phu Kradueng National Park, in evergreen forest, near Sam Kok Done, 16°52'30.63"N, 101°48'53.50"E, 1010 m alt., 5 Dec. 2014, *Tagane S., Nagamasu H., Naiki A., Rueangruea S., Suddee S., Okabe N, Keiwbang W., Hemmarat J., Supong W. T3469* [fr.] (BKF!, FU!); ibid., on the top plateau and edge of evergreen forest, 16°52'42.86"N, 101°42'57.20"E, 1288 m alt., 12 Jun. 2015, *Tagane S., Toyama H., Naiki A., Suddee S., Rueangruea S., Kanemitsu H., Keiwbang W., Hemmarat J. T4709* [fr.] (BKF!, FU!).

Distribution. Vietnam (Dong Nai - type locality, Lam Dong), Thailand (Loei).

Lithocarpus yersinii A.Camus, Bull. Mus. Natl. Hist. Nat., sér. 2, 6: 93 (1934); Ho, Ill. Fl. Viet. 2: 655, Fig. 6614 (2003).

Type. VIETNAM. Khanh Hoa Province, “Sud-Annam: Prov. de Nha-trang, massif de Honba”, 1000 m alt., 20–22 Sep. 1918, *Chevalier A.J.B 38904* [fr.] (holotype: P [P00744711!]; isotype: P [P00744712!]).

Additional specimens examined. VIETNAM. Khanh Hoa Province: Mt. Hon Ba, 12°07'8.64"N, 108°56'51.99"E, 1498 m alt., 17 Jul. 2013, *Tagane S., Yahara T., Nagamasu H., Fuse K., Toyama H., Tran H., Dang V.S., Loi X.N., Thach N.D., Cuong Q.N., Hieu P.N.H., Thach K.N. V65, V106* [ster.] (FU!, VNM!); ibid., 12°07'6.47"N, 108°56'46.38"E, 1521 m alt., 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. V1424* [ster.] (FU!, VNM!); ibid., 12°07'01.82"N, 108°56'46.63"E, 1545 m alt., 24 Nov. 2014, *Yang C.J., Toyama H., Tagane S., Dang V.S., Nagamasu H., Naiki A., Tran H., Hieu H.N.P., Cuong N.Q. V2129* [ster.] (FU!, VNM!). Thua Thien Hue Province: Bach Ma National Park, on the trail from the summit down to temple, in evergreen forest, 16°11'56.87"N, 107°51'26.01"E, 1412 m alt., 27 May 2015, *Yahara T., Tagane S.. Toyama H., Nguyen N., Nguyen C., Okabe N. V2991* [fl.] (DLU!, FU!). Lam Dong Province: Bi Doup Nui Ba National Park, in secondary lower montane evergreen forest, 12°11'14.81"N, 108°40' 24.28"E, 1544 m alt., 25 Feb. 2016, *Tagane S., Wai J. V4407* [ster.] (DLU!, FU!).

Distribution. Vietnam (Khanh Hoa - type locality, Lam Dong, Thua Thien Hue).

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Legends

Supplementary 5.S1. List of species and their voucher specimens used in the present study.

Complex	Species	Vouchers	Coordinate		Altitude (m)	Localities
			Latitude	Longitude		
Lithocarpus elegans complex	*L. blaoensis*	Ngoc et al. V3176 (DLU, FU)	11°43' 37.0"N	107°42' 34.5"E	1000	Lam Dong, Vietnam
		Yahara et al. V2995 (DLU, FU)	16°11' 56.87"N	107°51' 26.01"E	1412	Bach Ma NP, Vietnam
		Yahara et al. V3004 (DLU, FU)	16°11' 49.05"N	107°51' 22.43"E	1348	Bach Ma NP, Vietnam
		Tagane et al. V6524 (FU, DLU)	15°10'07.4"N	107°45'43.4"E	1376	Ngoc Linh NR, Vietnam
		Tagane et al. V6524 (FU, DLU)	15°12'24.2"N	107°46'10.4"E	1365	Ngoc Linh NR, Vietnam
		Ngoc et al. V6956 (FU, DLU)	15°59'51"N	107°59'17"E	1450	Ba Na NR, Vietnma
	L. bokorensis	Tagane et al. 4131 (FU, Cam)	10°38'12.59"N	104°02'06.37"E	1014	Mt. Bokor, Cambodia
	L. aff. *bokorensis*	Tagane et al. 4713 (FU, Cam)	11°32'10.21"N	103°09'46.85"E	183	Mt. Bokor, Cambodia
	L. elegans	Yahara et al. IJ796 (FU)	06°45' 54.94"S	106°58'58.83"E	2120	Gede, Indonesia
		Yahara et al. IJ822 (FU)	06°45' 54.94"S	106°58'58.83"E	2120	Gede, Indonesia
		Yahara et al. IJ1047 (FU)	06°46'22.12"S	106°58'35.84"E	2310	Gede, Indonesia
		Yahara et al. IJ1078 (FU)	06°46'22.12"S	106°58'35.84"E	2310	Gede, Indonesia
		Tagane et al. IJ1108 (FU)	06°46.557'S	107°58.607'E	2400	Gede, Indonesia
	L. grandifolius	Tagane et al. MY1552 (FU)	24°58'03.84"N	096°22'32.88"E	839	Indawgyi, Myanmar
	L. monoromensis	Ngoc et al. V3167 (DLU, FU)	11°54' 59.8"N	108°27' 27.8"E	1400	Lam Dong, Vietnam
		Ngoc et al. V5399 (DLU, FU)	11°12'23.1"N	107°04'06.6"E	89	Dong Nai, Vietnam
		Tagane et al. 6903 (FU, Cam)	12 24'43.32"N	107 11'04.78"E	668	Mondulkiri, Cambodia
		Tagane et al. 7277 (FU, Cam)	12°29'22.8"N	107°10'42.2"E	771	Mondulkiri, Cambodia
		Tagane et al. 7317 (FU, Cam)	12°29'22.8"N	107°10'42.2"E	771	Mondulkiri, Cambodia

		Tagane et al. 7617 (FU)	12°24'51.4"N	107°11'18.7"E	667	Mondulkiri, Cambodia
	L. syncarpus	Ngoc et al. V3188 (DLU, FU)	11°43' 37.0"N	107°42' 34.5"E	1000	Lam Dong, Vietnam
		Ngoc et al. V3246 (DLU, FU)	11°28'23.5"N	108°03'58.9"E	1100	Lam Dong, Vietnam
		Ngoc et al. V3250 (DLU, FU)	11°28'23.5"N	108°03'58.9"E	1100	Lam Dong, Vietnam
		Tagane et al. T3469 (BKF, FU)	16°52'30.63"N	101°48'53.50"E	1010	Phu Kradueng, Thailand
		Tagane et al. T4709 (BKF, FU)	16°52'42.86"N	101°42'57.20"E	1288	Phu Kradueng, Thailand
Relatives of the *L. elegans* complex	*L. harmandii* subsp. *harmandii*	Toyama et al. 379 (FU, Cam)	12°22'48.54"N	105°9' 43.59"E	32	Kampong Thom, Cambodia
		Toyama et al. 419 (FU, Cam)	12°22'48.47"N	105°9'40.93"E	31	Kampong Thom, Cambodia
		Toyama et al. 1023 (FU, Cam	12°22'48.75" N	105°9'17.32"E	26	Kampong Thom, Cambodia
	L. harmandii subsp. *malacotrichus*	Ngoc et al. V3171 (DLU, FU)	11°37' 44.3"N	108°16' 30.1"E	890	Lam Dong, Vietnam
		Yahara et al. L1818 (FOF, FU)	18 22'35.28"N	102 51'29.46"E	905	Phou Khao Khouay, Laos
		Yahara et al. L1835 (FOF, FU)	18 22'35.28"N	102 51'29.46"E	905	Phou Khao Khouay, Laos
		Yahara et al. L1841 (FOF, FU)	18 22'35.28"N	102 51'29.46"E	905	Phou Khao Khouay, Laos
Lithocarpus hancei complex	*L. hancei*	Nguyen et al. V4800 (DLU, FU)	22°20'57.1"N	103°46'15.4"E	1900	Hoang Lien NP, Vietnam
		Nguyen et al. V5111 (DLU, FU)	22°19'48.0"N	103°46'57.5"E	2225	Hoang Lien NP, Vietnam
		Nguyen et al. V4924 (DLU, FU)	22°20'57.1"N	103°46'15.4"E	1900	Hoang Lien NP, Vietnam
		Yahara et al. V5833 (DLU, FU)	18°11'41.7"N	105°23'06.1"E	1726	Vu Quang NP, Vietnam
	L. jacsonianus	Nguyen et al. V4918 (DLU, FU)	22°21'04.3"N	103°46'20.7"E	1919	Hoang Lien NP, Vietnam
		Nguyen et al. V5345 (DLU, FU)	22°19'47.5"N	103°49'18.0"E	1288	Hoang Lien N, Vietnam
		Nguyen et al. V5441 (DLU, FU)	19°00'20.3"N	104°48'10.9"E	400	Pumat NP, Vietnam
		Bon et al. V6043 (FU)	21°03'43.4"N	105°21'39.7"E	1125	Ba Vi NP, Vietnam
		Bon et al. V6051 (FU)	21°03'43.4"N	105°21'39.7"E	1125	Ba Vi NP, Vietnam

		Bon et al. V6062 (FU)	21°03'43.4"N	105°21'39.7"E	1125	Ba Vi NP, Vietnam
		Yahara et al. V7432 (DLU, FU)	21°03.622"N	105°21.747''E	1161	Ba Vi NP, Vietnam
		Yahara et al. V7433 (DLU, FU)	21°03.622"N	105°21.747''E	1161	Ba Vi NP, Vietnam
		Tagane et al. V3092 (DLU, FU)	16°00'07.30"N	108°01'33.90"E	707	Ba Na NR, Vietnam
		Tagane et al. V6261 (DLU, FU)	15°10'5.7"N	107°45'23.58"E	1067	Ngoc Linh NR, Vietnam
		Tagane et al. V6400 (DLU, FU)	15°10'5.7"N	107°45'23.58"E	1376	Ngoc Linh NR, Vietnam
	L. yersinii	Tagane et al. V65 (FU, VNM)	12°07'8.64"N	108°56' 51.99"E	1498	Hon Ba NR, Vietnam
		Tagane et al. V106 (FU, VNM)	12°07'8.64"N	108°56' 51.99"E	1498	Hon Ba NR, Vietnam
		Toyama et al. V1424 (FU, VNM)	12°07'6.47"N	108°56'46.38"E	1521	Hon Ba NR, Vietnam
		Yang et al. V2129 (FU, VNM)	12°07'01.82"N	108°56'46.63"E	1545	Hon Ba NR, Vietnam
		Yahara et al. V2991 (DLU, FU)	16°11'56.87"N	107°51'26.01"E	1412	Bach Ma NP, Vietnam
		Tagane et al. V4407 (DLU, FU)	12°11'14.81"N	108°40' 24.28"E	1544	Bidoup-Nui Ba NP, Vietnam
Additional taxa	*L. balansae*	Toyama et al. V1233 (FU)	12° 6'39.77"N	108°58'59.23"E	617	Hon Ba NR, Vietnam
		Yahara et al. V2697 (DLU, FU)	16°12' 05.03"N	107°50' 52.22"E	1000	Bach Ma NP, Vietnam
		Yahara et al. V2709 (DLU, FU)	16°12' 05.03"N	107°50' 52.22"E	1000	Bach Ma NP, Vietnam
		Yahara et al. V2938 (DLU, FU)	16°12' 17.19"N	107°51' 46.26" E	838	Bach Ma NP, Vietnam
		Nguyen et al. V3177 (DLU, FU)	11°43' 37.0"N	107°42' 34.5"E	1000	Lam Dong, Vietnam
		Nguyen et al. V5512 (DLU, FU)	19°00'20.3"N	104°48'10.9"E	400	Pumat NP, Vietnam
		Nguyen et al. V5447 (DLU, FU)	19°00'20.3"N	104°48'10.9"E	400	Pumat NP, Vietnam
		Tagane et al. V6125 (DLU, FU)	15°10'05.7"N	107°45'23.6"E	1067	Ngoc Linh NR, Vietnam
		Tagane et al. V6397 (DLU, FU)	15°10'07.4"N	107°45'43.4"E	1376	Ngoc Linh NR, Vietnam
	L. litseifolius	Tagane et al. V6602 (DLU, FU)	15°11'55.7"N	107°46'14.3"E	1354	Ngoc Linh NR, Vietnam

		Tagane et al. V6667 (DLU, FU)	15°12'24.2"N	107°46'10.4"E	1365	Ngoc Linh NR, Vietnam
		Tagane et al. V6756 (DLU, FU)	15°11'57.9"N	107°47'07.8"E	1265	Ngoc Linh NR, Vietnam
		Tagane et al. V6757 (DLU, FU)	15°11'57.9"N	107°47'07.8"E	1265	Ngoc Linh NR, Vietnam
	L. petelotii	Yahara et al. V2797 (DLU, FU)	16°11' 44.20"N	107°51' 20.58"E	1285	Bach Ma NP, Vietnam
		Yahara et al. V2821 (DLU, FU)	16°11' 44.20"N	107°51' 20.58"E	1285	Bach Ma NP, Vietnam
		Yahara et al. V5778 (DLU, FU)	18°11'34.9"N	105°23'08.9"E	1634	Vu Quang NP, Vietnam
		Yahara et al. V5801 (DLU, FU)	18°11'28.2"N	105°23'13.2"E	1587	Vu Quang NP, Vietnam
	L. pleiocarpus	Nguyen et al. V4951 (DLU, FU)	22°20'55.5"N	103°46'18.1"E	1910	Hoang Lien NP, Vietnam
	L. rouletii	Tagane et al. V317 (FU)	12°07'8.6"N	108°56'51.9"E	1498	Hon Ba NR, Vietnam
		Tagane et al. V590 (FU)	12°07'29.45"N	108°57'51.11"E	1204	Hon Ba NR, Vietnam
		Toyama et al. V1074 (FU)	12°6'46.88"N	108°58'14.43"E	919	Hon Ba NR, Vietnam
		Tagane et al. V1754 (FU)	12°07'06.47"N	108°56'51.99"E	1521	Hon Ba NR, Vietnam
		Toyama et al. V2174 (FU)	12°07'11.42"N	10857'25.76"E	1336	Hon Ba NR, Vietnam
		Nguyen et al. V3516 (DLU, FU)	18°16'39.6"N	105°22'20.1"E	115	Vu Quang NP, Vietnam
		Nguyen et al. V3780 (DLU, FU)	18°15'48.3"N	105°20'58.6"E	914	Vu Quang NP, Vietnam
Outgroup	*C. cerebrina*	Ngoc et al. V5503 (DLU, FU)	19°00'20.3"N	104°48'10.9"E	400	Pumat NP, Vietnam
	C. piriformis	Tagane et al. V6689 (DLU, FU)	15°11'11.8"N	107°50'36.3"E	833	Ngoc Linh NR, Vietnam
	Q. helferina	Ngoc et al. V3169 (DLU, FU)	11°54'59.8"N	108°27'27.8"E	1400	Lam Dong, Vietnam
	Q. langbianensis	Tagane et al. V3962 (DLU, FU)	12°10'34.7"N	108°41'08.4"E	1533	Bidoup-Nui Ba NP, Vietnam
	Q. poilanei	Yahara et al. V2986 (DLU, FU)	16°11'56.87"N	107°51'26.01" E	1412	Bach Ma NP, Vietnam

Table 5.1. The morphological traits of each species of *L. elegans* complex, *L. harmandii* and *L. harmandii* subsp. *malacotrichus*

Taxa	Leaves	Petiole length	No. of Secondary veins	Infruct. length	Cupule	Scales arrangement	Nut	Nut enclosure	Basal scar of the nut
L. blaoensis[1,2,3]	Ovate, 11–16 × 3–5 cm, base cuneate, glabrous on both sides.	2–2.5 cm long	10–12 pairs	9–14 cm long	Bowl or cup shape, 7–10 mm tall × 17–20 mm in diam.	Imbricate	Depressed ovoid or turbinate, 17–19 mm tall × 17–18 mm in diam.	1/2 or more than 1/2 of the nut	Concave, 10–12 mm in diam.
L. bokorensis[3]	Blade obovate, oblong-elliptic, elliptic, 3.7–13 × 2.1–4.8 cm, base cuneate to attenuate, glabrous on both sides.	0.2–1.1 cm long	8–10 pairs	7–8.5 cm long	Broadly saucer-shaped, 5 mm tall × 12–15 mm in diam.,	Imbricate	Broadly ovoid, depressed at both ends, 10–12 mm tall × 13–14 mm in diam.	1/5–1/3 of the nuts	Concave, ca. 8 mm in diam
***L. elegans* s. str.**[1,2,3]	Narrowly to broadly obovate or elliptic, 6.5–27 × 3–11.5 cm, base cuneate, sometime subrounded or acute, glabrous on both sides.	0.5–1 cm long	9–13 pairs	15–30 cm long	Cup- to saucer-shaped, 10–15 m tall × 20–25 mm in diam.	Imbricate to more or less concentrically set	Ovoid or depressed ovoid to sub-globose, 15–20 mm tall × 20–25 mm in diam.	1/4–1/2 of the nut	Flat or concave, 10–15 mm in diam.
L. grandifolius[1,2,3]	Narrowly oblong-elliptic to oblanceolate, 6.3–15 × 2.2–5.3 cm, base cuneate to attenuate, glabrous.	1.5–4 cm long	9–13 pairs	15–20 cm long	Saucer-shaped, ca. 6–8 mm tall × 15–20 mm in diam.	Connate into 5–7 horizontal ridges	Ovoid, acute apex and flat at basal, 17-20 × 15–18 mm in diam.	enclosing 1/7–1/6 of nut	Concave, 8–12 mm in diam.
L. monoromensis[3]	Narrowly lanceolate to oblanceolate, 6.3–15 × 2.2–5.3 cm, base attenuate, glabrous.	0.5–1.3 cm long	11–13 pairs	19–26 cm long	Saucer-shaped, 3–4 mm tall × 13–15 mm in diam.	Imbricate	Depressed ovoid, 15–17 mm tall × 14–18 mm in diam.	1/7 of the nut	Concave, ca. 11–14 mm in diam.
L. syncarpus[1,2,3]	Ovate-lanceolate, 10–19 × 2.5–8 cm, base cuneate, glabrous on both sides.	1.5–2.5 cm long	11–13 pairs	10–16 cm long	Saucer-shaped, 4–5 mm tall × 17–23 mm in diam.	Imbricate	Depressed globose and flat at basal,	Less than 1/4 of the nut	Concave, 12–15 mm in diam.

							12–13 mm tall × 15–17 mm in diam.		
L. harmandii[1,2,3]	Ovate-lanceolate, 12–25 × 10–13 cm, base attenuate, yellow pubescent beneath.	1.5–4 cm long	8–12 pairs	15–30 cm long	Saucer-shaped, 4–5 mm tall × 10–12 mm in diam.	Imbricate	Obovoid, subovoid, 16–17 mm tall × 15–16 mm in diam.	Less than 1/3 of the nuts	Concave, 6–7 mm in diam.
L. harmandii subsp. *malacotrichus*	Broad elliptic, 15–27 × 8–15 cm, base obtuse, densely white tomentose on both sides.	2.5–3.5 cm long	8–13 pairs	21–25 cm long	Cup-shape or saucer-shaped, 5–7 mm tall × 10–12 mm in diam.	Imbricate	Elongate turbinate, glabrous, 16–18 mm tall × 10–13 mm in diam.	1/3 of the nut	Flat, sometimes convex at central, ca. 6–7 mm in diam.

[1] Derived from original description; [2] Derived from type materials and herbaria collections, [3] Derived from this study collection.

Table 5.2. The morphological traits of each species of *L. hancei* complex

Taxa	Leaf size	Petiole length	No. of secondary veins	Infruct. length	Cupule	Scales arrangement	Nut size	Nut enclosure	Basal scar of the nut
L. hancei[1,2,3]	5–10 × 2.5–5 cm	1.5–4 cm long	6–13 pairs	6–8 cm long	Clustered of 3–5, 3–7 mm × 10–20 mm in diam.	Imbricate	8–20 mm tall × 6–25 mm in diam.	Enclosing nearly 1/3 of nut	Concave, 5–10 mm in diam
L. jacsonianus[1,2,3]	8–11 × 2.5–3 cm	1.2–1.5 cm long	10–11 pairs	8–11 cm long	Crowded, Clustered of 3, 3–4 mm × 8–10 mm in diam.	Imbricate	9–12 mm tall × 10–12 mm indiam.	Basal to 1/4 of the nut	Comcave, 6–8 mm in diam.
L. sabulicolus[1,2]	6–8 × 3.5–5 cm	1 cm long	8–10 pairs	12–14 cm long	Solitary, 5–6 mm × 15–16 mm in diam.	Imbricate	15–18 mm tall × 15 mm in diam.	1/4 of the nut	Concave, ca. 6 mm in diam.
L. yersinii[1,2,3]	7–10 × 3–4 cm	1.2–1.5 cm long	8–10 pairs	8–10 cm long	Solitary, 8–10 × 10–12 mm in diam.	Forming 3–4 concentric rings	18–20 mm tall × 10–15 mm in diam.	1/3 of the nut	Concave, ca. 5 mm in diam.

[1] Derived from original description; [2] Derived from type materials and herbaria collections, [3] Derived from this study collection.

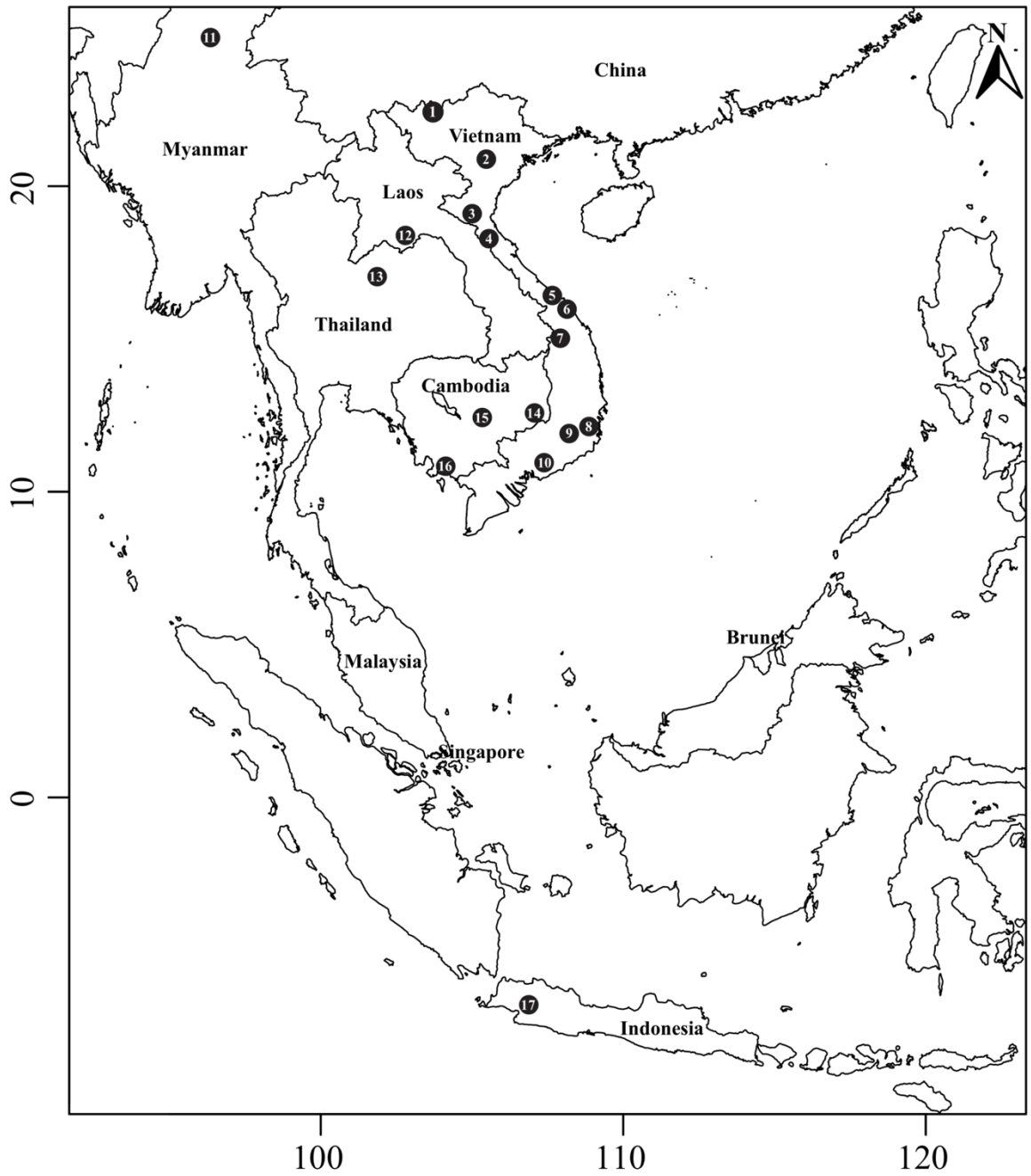

Figure 5.1. Collection sites in this study: (**1**) Hoang Lien NP, (**2**) Ba Vi NP, (**3**) Pu Mat NP, (**4**) Vu Quang NP, (**5**) Bach Ma NP, (**6**) Ba Na NR, (**7**) Ngoc Linh NR, (**8**) Hon Ba NR, (**9**) Bidoup – Nui Ba NP, Lam Dong Province, (**10**) Dong Nai NR, **(11)** Indawgyi Wildlife Sanctuary, (**12**) Phou Khao Khouay National Protected Area, (**13**) Phu Kradueng NP, (**14**) Seima Protected Forest, (**15**) Kampong Chhnang, (**16**) Bokor NP, (**17**) Gede Pangorango NP. The map was generated and modified by Ngoc Nguyen using RStudio ver. 1.1.383 (mapdata packages, RStudio, Inc.) and Adobe Illustrator CC 2017 ver. 21.0.0 (Adobe Systems, San Francisco, CA, USA).

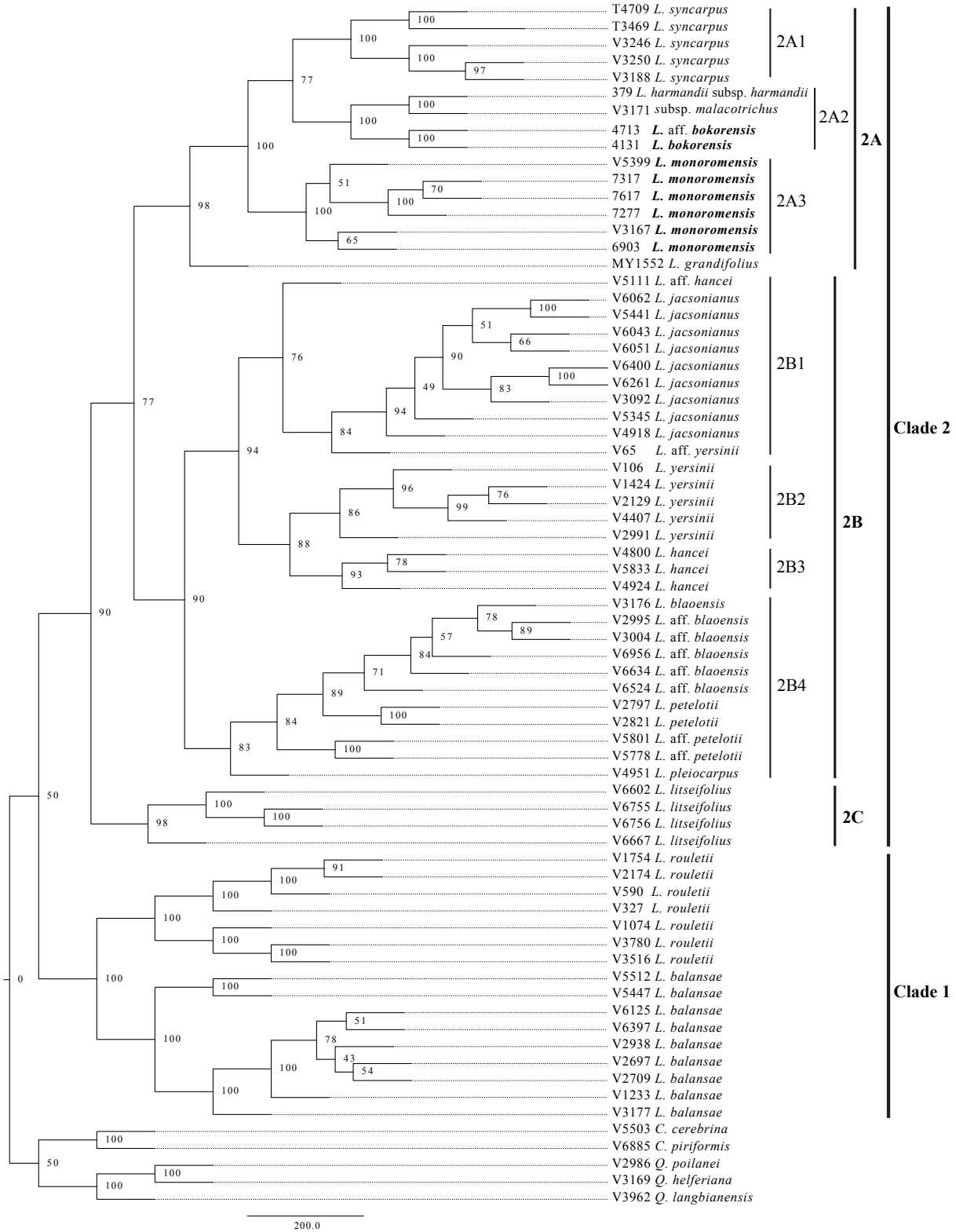

Figure 5.2. Neighbor-joining tree using 66 samples of *Lithocarpus* and outgroup based on presence/absence data of 34,449 MIG-seq loci. Branches are labeled with bootstrap support (% of 1000 replicates).

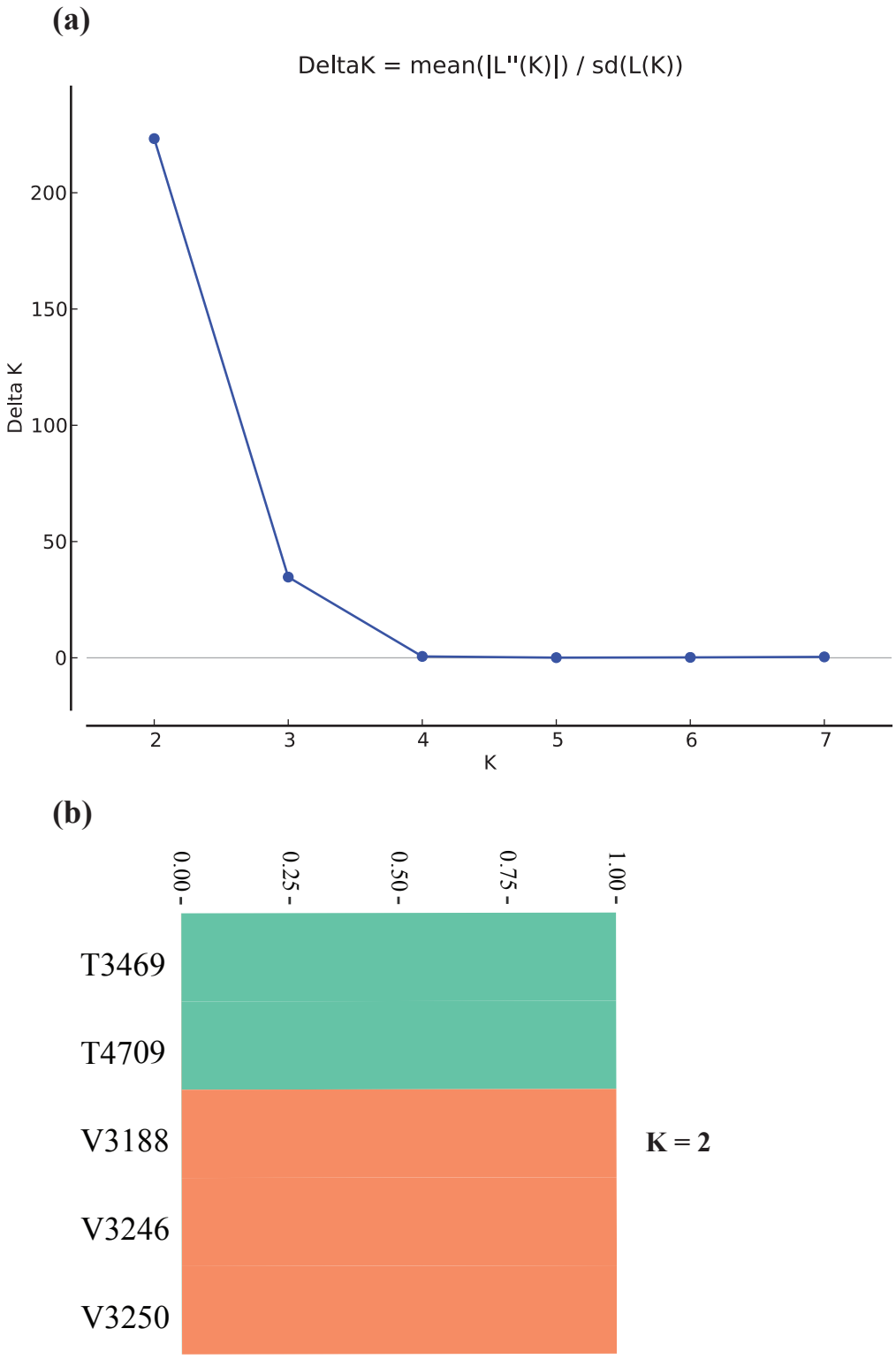

Figure 5.3. Population genetic structure analysis of *L. syncarpus*: (a) The optimal K value (b) Bar plot of population genetic structure.

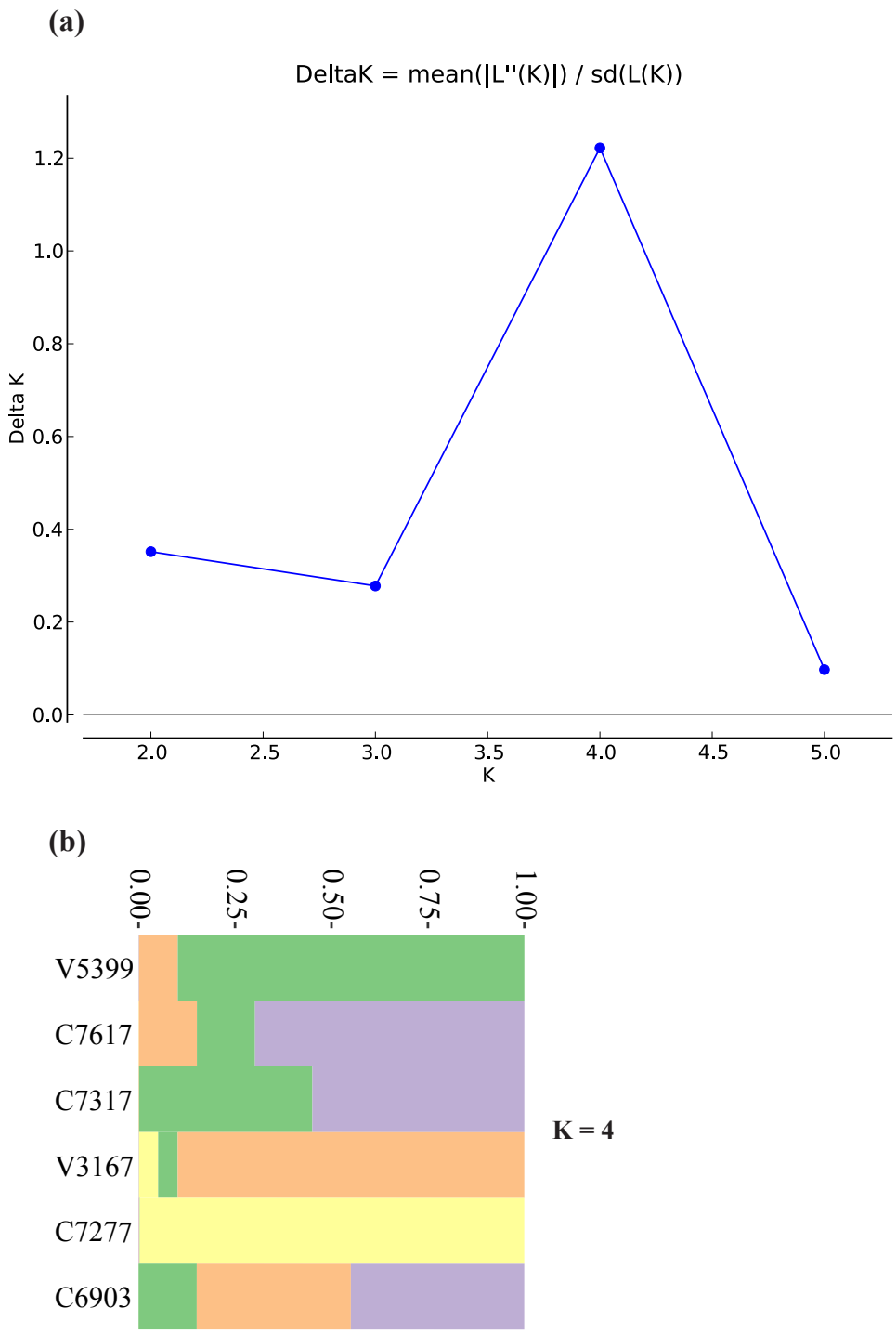

Figure 5.4. Population genetic structure analysis of *L. monoromensis*: (a) The optimal K value (b) Bar plot of population genetic structure.

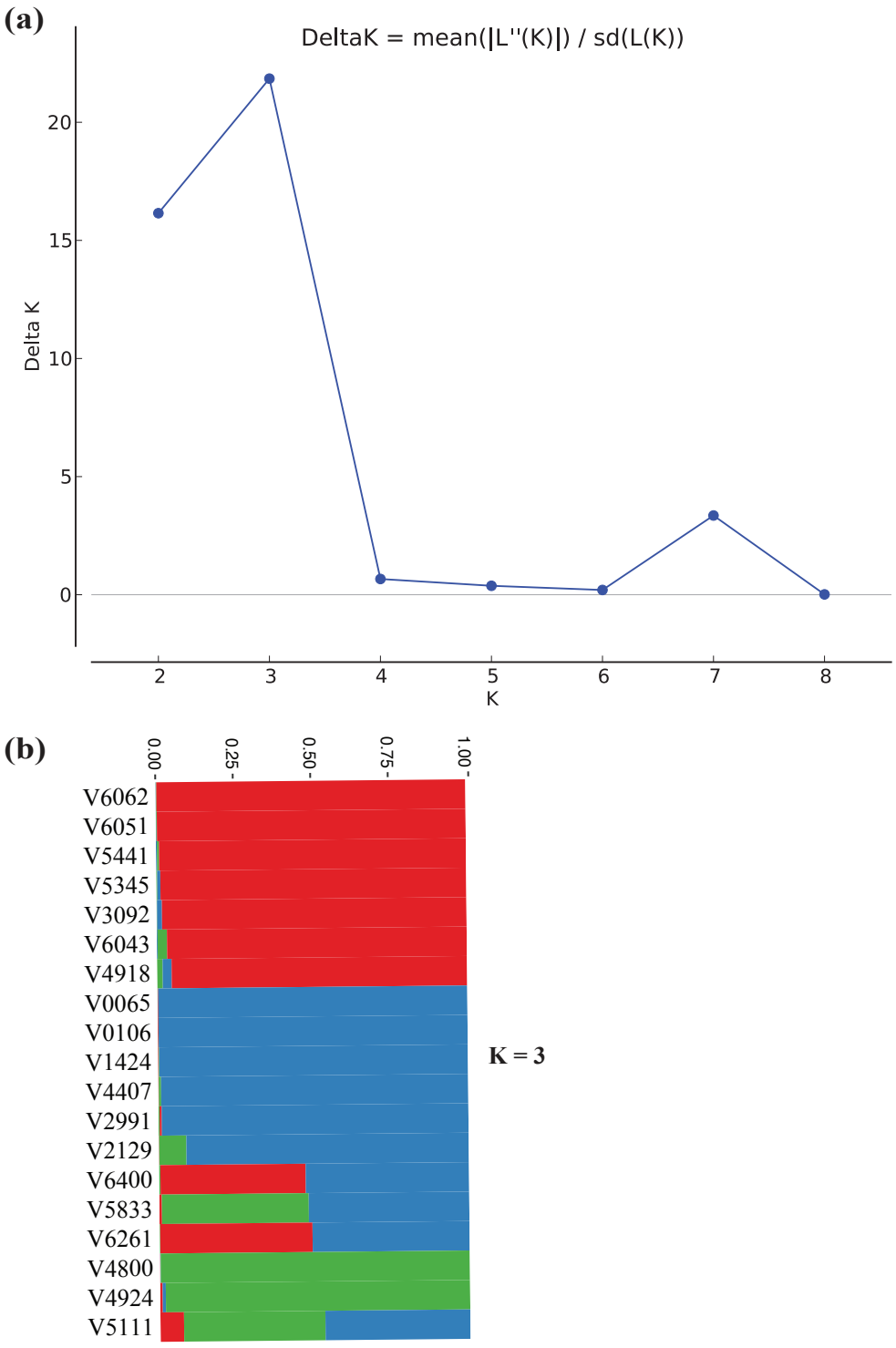

Figure 5.5. Population genetic structure analysis of *L. hancei* complex: (a) The optimal K value (b) Bar plot of population genetic structure.

Figure 5.6. *Lithocarpus bokorensis* Ngoc, Tagane & Yahara: (**a**) Holotype (KYO), (**b**) Leafy twig, (**c**) Abaxial leaf surface, (**d**) Male inflorescences, (**e**) Infructescences, (**f**) Mature acorn, (**g**) basal scar of nut. Scale bars (**c**) = 5 cm, (**d, f, g**) = 1 cm, (**e**) = 2 cm. Materials from *Tagane et al. 4131* (FU).

Figure 5.7. *Lithocarpus monoromensis* Ngoc, Tagane & Yahara: (**a**) Holotype (KYO), (**b**) Leafy twig with young infructescences, (**c**) Abaxial leaf surface, (**d**) Infructescences, (**e**) Mature acorn, (**g**) Top and bottom view of nuts. Scale bars (**c, d**) = 5 cm, (**e**) = 1 cm. Materials from *Tagane et al. 7317* (FU).