

MICROSCOPIC AND MOLECULAR ANALYSES OF SELECTED *STRYCHNOS* SPECIES

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บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)  
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย

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A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science Program in Public Health Sciences  
College of Public Health Sciences  
Chulalongkorn University  
Academic Year 2015  
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ลักษณะทางจุลทรรศน์และอนุพันธุศาสตร์ของพืชสกุลสตรีกโนส



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ปีการศึกษา 2558

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

Thesis Title	MICROSCOPIC AND MOLECULAR ANALYSES OF SELECTED <i>STRYCHNOS</i> SPECIES
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ขนิษฐา นาคเกลี้ยง : ลักษณะทางจุลทรรศน์และอนุพันธุศาสตร์ของพืชสกุลสตรีกโนส (MICROSCOPIC AND MOLECULAR ANALYSES OF SELECTED *STRYCHNOS* SPECIES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. กาญจนา รังษีหิรัญรัตน์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. นิจศิริ เรืองรังษี, หน้า.

พืชสกุลสตรีกโนส 4 ชนิดที่พบในประเทศไทย ได้แก่ *Strychnos thorelii*, *S. lucida*, *S. nux vomica* และ *S. nux-blanda* เป็นพืชสมุนไพรที่สำคัญในการแพทย์พื้นบ้าน พืชสกุลสตรีกโนสมิฤทธิ์แก้ไข้ แก้ปวด แก้พิษงู และรักษาโรคไขข้อ เนื่องจากพืชสกุลสตรีกโนส มีลักษณะทางพฤกษศาสตร์และชื่อพื้นถิ่นที่คล้ายคลึงกัน ทำให้เกิดการใช้แทนที่โดยไม่ได้ตั้งใจ การศึกษาลักษณะทางจุลทรรศน์ และค่าคงที่ของใบ (จำนวนปากใบ ดัชนีปากใบ อัตราส่วนแฟลลิสเตด จำนวนเส้นปลายใบ จำนวนเซลล์ผิวใบและพื้นที่ของเซลล์ผิวใบ) ทั้ง 4 ชนิด พบปากใบชนิด paracytic ไม่พบขนทั้งด้านบนและด้านล่างของใบ การหาคงที่ของใบระหว่างสตรีกโนส 4 ชนิดพบว่าสามารถใช้จำแนกความแตกต่างของพืชสกุลสตรีกโนสได้ โดยเฉพาะอย่างยิ่งดัชนีปากใบ ซึ่งพบค่าสูงสุดใน *S. nux-blanda* (ช่วง 15.24-16.44) และต่ำสุดใน *S. lucida* (ช่วง 6.77-7.52) การศึกษาลำดับนิวคลีโอไทด์ของบริเวณ ITS ยีน *rbcL* และยีน *matK* พบความยาวของลำดับนิวคลีโอไทด์ประมาณ 700 1400 และ 1800 ตามลำดับ ความเหมือนของลำดับนิวคลีโอไทด์ของทั้ง 3 บริเวณภายในพืชชนิดเดียวกัน มีค่าร้อยละ 95 ถึง 99 และความเหมือนของลำดับนิวคลีโอไทด์ระหว่างพืชต่างชนิดมีค่าร้อยละ 87 ถึง 99 เทคนิคพีซีอาร์-อาร์เอฟแอลพี (PCR-RFLP) ของยีน *matK* ถูกพัฒนาขึ้น เมื่อตัดด้วยเอนไซม์ตัดจำเพาะ *DraI* และ *XbaI* สามารถจำแนกสตรีกโนสทั้ง 4 ได้ โดยดูจากความแตกต่างของขนาดชิ้นดีเอ็นเอ ผลการศึกษาสรุปได้ว่าลักษณะทางจุลทรรศน์และอนุพันธุศาสตร์สามารถใช้ในการจำแนกพืชสกุลสตรีกโนสทั้ง 4 ชนิดที่พบในประเทศไทยได้

สาขาวิชา วิทยาศาสตร์สาธารณสุข

ลายมือชื่อนิสิต .....

ปีการศึกษา 2558

ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

# # 5778952453 : MAJOR PUBLIC HEALTH SCIENCES

KEYWORDS:

KANITTHA NAKKLIANG: MICROSCOPIC AND MOLECULAR ANALYSES OF SELECTED *STRYCHNOS* SPECIES. ADVISOR: ASST. PROF. KANCHANA RUNGSIHIRUNRAT, Ph.D., CO-ADVISOR: ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., pp.

There are four *Strychnos* species in Thailand including *Strychnos thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* which presented as important medicinal plants in folk medicine. *Strychnos* species have several ethnobotanical uses, they play an important role against fever, pain, antidote for snake poisoning and rheumatism. Many *Strychnos* species possess the similar morphology and vernacular name resulting in unintentional substitution. This current research aimed to investigate leaf microscopic characteristics and leaf measurement (stomatal number, stomatal index, palisade ratio, veinlet termination number, epidermal cell number and epidermal cell area) among four *Strychnos* species. The results showed paracytic type of stomata. There are no trichomes or cicatrices on both adaxial and abaxial epidermis. The leaf constants among four species are able to differentiate the *Strychnos* species, especially stomatal index which is highest in *S. nux-blanda* (range 15.24-16.44) and lowest in *S. lucida* (range 6.77-7.52). The full-length of nucleotide sequencing ITS region, *rbcl* and *matK* gene were also evaluated and the result showed the sequence length in ITS region, *rbcl* and *matK* gene were approximately 700, 1400 and 1800 base pairs respectively. The intra-species of three sequence shown 95-99% similarity and the inter-species of three sequence shown 87-99% similarity. PCR-RFLP method based on the *matK* gene was developed. After digestion with specific restriction enzyme using *DraI* and *XbaI*, four *Strychnos* species were easily distinguished based on the different sizes of the digested fragments. In conclusion, the use of both microscopic and molecular analyses are successful for the identification of *Strychnos* species in Thailand.

Field of Study: Public Health Sciences Student's Signature .....

Academic Year: 2015 Advisor's Signature .....

Co-Advisor's Signature .....

## ACKNOWLEDGEMENTS

The author wishes to express her heartily gratitude and appreciation to her thesis advisor, Assistant Professor Dr. Kanchana Rungsahirunrat, for her guidance, suggestion, and support throughout the course of this study.

The author is sincerely grateful to her thesis co-advisor, Associate Professor Dr. Nijisiri Ruangrunsi, for his kindness and valuable suggestion to complete the study.

Gratitude is grateful to the thesis committee members, Dr. Orawan Theanphong, Assistant Professor Dr. Chanida Palanuvej and Assistant Professor Dr. Naowarat Kanchanakhan for their important and constructive suggestion in finalizing this thesis.

This research was supported by Research Assistant Scholarship, Chulalongkorn University. The authors appreciate all staff member of College of Public Health Sciences, Chulalongkorn University, Thailand for instrument supports.

The author's final thank goes to her family, especially her dear parents for their love, encourage, and support her to concentrate on her study.

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## LIST OF ABBREVIATIONS

°C	degree celsius
M	molarity
mg	milligram
MgCl <sub>2</sub>	magnesium chloride
min	minute
ml	milliliter
mm	millimeter
mM	millimolarity
mm <sup>2</sup>	square millimeter
ng	nanogram
nm	nanometer
UV	ultraviolet
rpm	round per minute
SD	standard deviation
μl	microlitre
μm	micrometer
μM	micromolar
bp	base pair
cm	centimeter
DNA	deoxyribonucleic acid
Avg	average



## LIST OF ABBREVIATIONS

DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphates (dATP, dTTP, dGTP, dCTP)
ddNTP	dideoxyribonucleotide triphosphates (ddATP, ddTTP, ddGTP, ddCTP)
EDTA	ethylenediaminetetraacetic acid
RFLP	restriction fragment length polymorphism
<i>Taq</i>	<i>Thermus aquaticus</i>
TBE buffer	tris-boric and EDTA buffer
PCR	polymerase chain reaction
A, T, C, G	nucleotide containing the base adenine, thymine, cytosine, and guanine, respectively

## CHAPTER I

### INTRODUCTION

World Health Organization (WHO) estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care including Thailand. There are various herbal plants are utilized as long as in Thai traditional medicine. The genus *Strychnos*, the largest genus of family Loganiaceae (Strychnaceae) [1] comprises about 200 species range from forest lianas to shrubs and trees, and can be subdivided into three geographically separated groups. There are at least 73 species of the South and Central America, 75 species of Africa and 44 species in Asia including Australia [2] Smitinand recorded the presence of 15 species in Thailand [3] including *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* which presented as important medicinal plants in folk medicine. *Strychnos* species have several ethnobotanical uses. A few species are well known as arrow poison and also ordeal poison. However in ethnobotanical usage, they play an important role against fever, rheumatism, pain and antidote for snake poisoning [4-5]. The dried seeds of *Strychnos nux-vomica* contain 2.6-3% of total alkaloids. Major compounds are strychnine and brucine and other poisonous compounds which are responsible to pharmacological and toxicological activities.

There was the case of unintentional substitution of medicinal herbs from *Strychnos* species because many *Strychnos* species possess similar morphology and same vernacular name [6]. *Strychnos nux-vomica* has similar botanical characteristics with their closely related species; *S. nux-blanda*, and *S. lucida*. As a result, authentication is fundamental for standardization of herbal medicine. There are many methods used for examination of medicinal plant, ranging from simple morphological examination to physicochemical analysis, and DNA molecular method. Each method has their drawbacks and advantages. Sometimes two or several methods are applied for primary authentication. Macroscopic and microscopic examinations are still the most practical and standard method used for primary herbal plants authentication due to its simple, rapid and inexpensive. Macroscopic identities are based on the

authentication of their gross morphological characteristics and organoleptic properties such as size, shape, color, flowers or fruit that are visible with naked eye. Microscopic examination is also the method most commonly used to authenticate herbal medicine by observing cell structure and internal features under the microscope. Microscopic examination focuses on anatomical and histological structures of plant materials such as powdered drug characteristics, transverse section characteristics of leaf midrib and leaf constant characteristics; palisade cell, trichomes (hair), the arrangement of stomata in epidermis or the presence/absence of accumulated compounds. Quantitative measurements of their cell numbers offer specific leaf constants [7]. Their characteristics are capable to identify and authenticate plants species leading to plant material quality control.

In difficult or critical cases, some medicinal plants are not easily characterized by microscopy analysis because the adulterant may lead to confuse or misunderstand by authentication. In addition, a complementary with other analytical molecular methods provides important supporting evidence [8]. DNA-based molecular techniques have been proved to be the powerful way to discriminate species with high accuracy because DNA characteristics is the unique heredity of each species and is not affected from environmental factors. Plant genomes are more complex than other eukaryotic organisms due to present of multiple chromosome; nuclear genome, chloroplast genome and mitochondrial genome. A specific region of DNA sequence in plant genome has been used as a modern genomics tool for herbal plant identification. An internal transcribed spacer (ITS) region of nuclear ribosomal DNA is the most commonly used sequence for plant identification [9]. Many chloroplast, mitochondrial and nuclear gene have been utilized for studying sequence variation at genus/species level [10]. However, there are slightly previous studies and the data are still limited about *Strychnos* species existing in Thailand. As a result, it is essential for studying the pharmacognostic characteristics and the genetic information of *Strychnos* species. The information generate from this work can be useful for species identification and developing the more reproducible and robust PCR-RFLP for rapid identification.

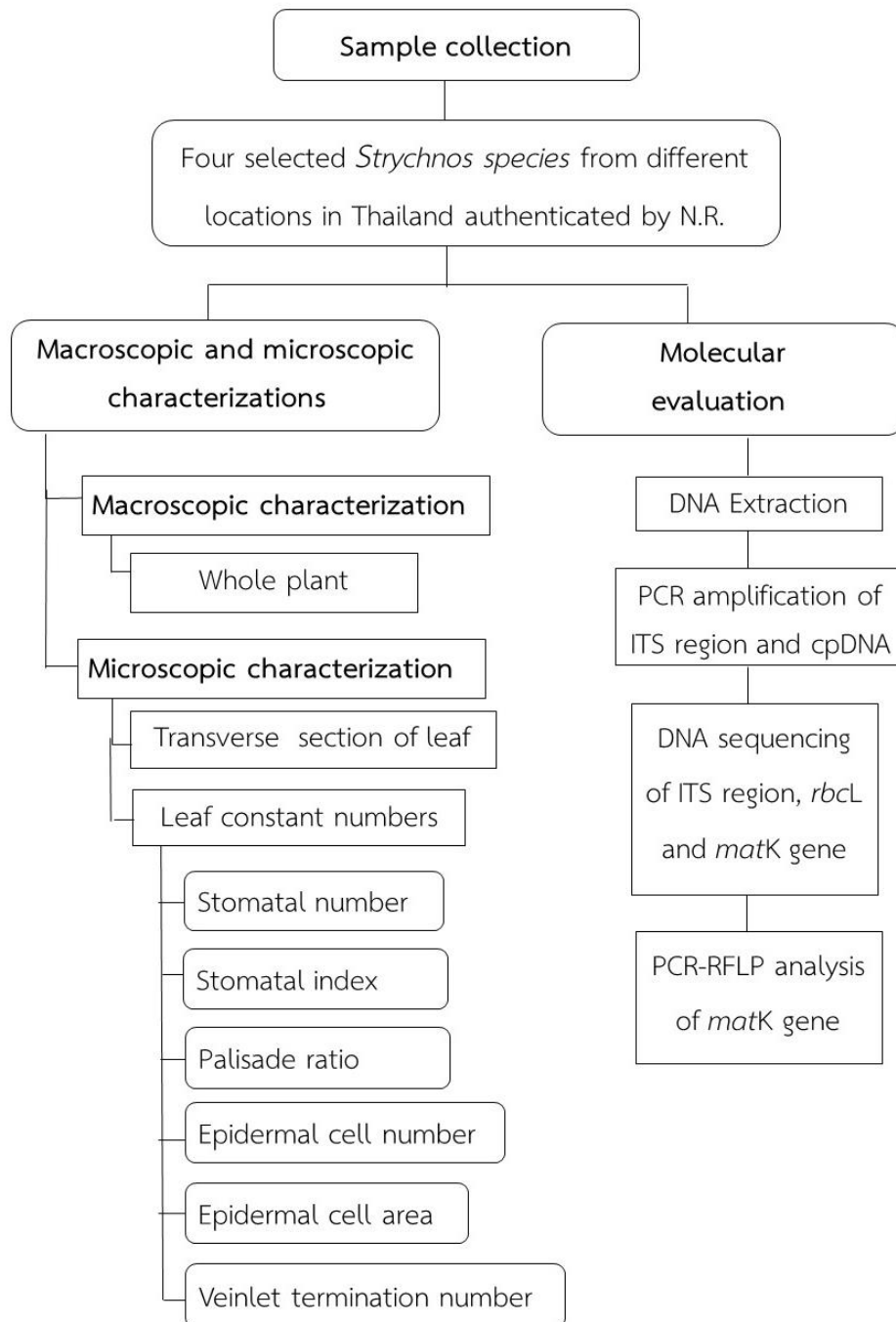
### Research questions

1. Do the values of leaf constant numbers; stomatal number, stomatal index, palisade ratio, veinlet termination number, epidermal cell number and epidermal cell area of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* is useful to identify each species?
2. Do the differences sequence of ITS region, maturase K (*matK*) gene and the large subunit of the ribulose-bisphosphate carboxylase (*rbcl*) gene of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* can be used to authenticate each species?
3. Does PCR-RFLP of *matK* gene can be used as a species-specific marker of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda*?

### Objectives of the study

1. To study the leaf constants; stomatal number, stomatal index and palisade ratio, veinlet termination number, epidermal cell number and epidermal cell area of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda*
2. To study the sequence variation of ITS region, maturase K (*matK*) gene and the large subunit of the ribulose-bisphosphate carboxylase (*rbcl*) gene of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda*.
3. To develop the PCR-RFLP method for species-specific marker of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda*.

## The conceptual framework



## CHAPTER II

### REVIEW OF RELATED LITERATURE

#### The genus *Strychnos*

*Strychnos* is a genus of flowering plants, belonging to family Loganiaceae (Strychnaceae). This genus comprises about 200 species range from forest lianas to shrubs and trees, all of which are pantropical in distribution and can be subdivided into three geographically separated groups of species. There are at least 73 species which are native of the south and Central America, 75 species of Africa and 44 species of Asia including Australia [2]. The only exception is *Strychnos potatorum* L. which is found both in Africa and Asia. Smitinand recorded the presence of 15 species of *Strychnos* in Thailand [3]. There are various Thai names for each species (the well-known vernacular name of each species are in bold printed) as shown in Table 1.

**Table 1** List of 15 *Strychnos* species and their vernacular name found in Thailand

No.	<i>Strychnos</i> species	Vernacular name
1	<i>Strychnos axillaris</i> Colebr.	khwak kai (Chaiyaphum), kho bet (Nong Khai), <b>khi raet</b> (Prachin Buri), khiao ngu (Chumphon), tueng khrua dam tua mae (Lampang), ben (Northeastern), ben kho (Northeastern), lep khrut (Chanthaburi), lep rok (Phatthalung), nam khem (Chaiyaphum), mak ta kai (Loei)
2	<i>Strychnos cathayensis</i> Merr.	-
3	<i>Strychnos curtisii</i> King & Gamble	<b>Kluai khieo</b>
4	<i>Strychnos kerrii</i> A. W. Hill = <i>Strychnos nitida</i> G. Don	kluai khiao (Nakhon Ratchasima), <b>san di lok</b> (Chiang Mai)
5	<i>Strychnos krabiensis</i> A. W. Hill = <i>Strychnos ignatii</i> P. J. Bergius	<b>phaya mue lek</b> (Krabi)
6	<i>Strychnos lanata</i> A. W. Hill	- กล้วย
7	<i>Strychnos lucida</i> R. Br.	<b>phaya mue lek</b> (Central), phaya mun lek (Central), ya mue lek (Krabi), siao duk (Northern), Strychnine bush
8	<i>Strychnos minor</i> Dennst.	tumka khao (Lampang), <b>tumka daeng</b> (Central, Northeastern, Lampang), thao kwang du thuk (Surat Thani), thao plong (Ranong)

**Table 1** List of 15 *Strychnos* species and their vernacular name found in Thailand

No.	<i>Strychnos</i> species	Vernacular name
9	<i>Strychnos nux-blanda</i> A. W. Hill	klo-wo-sae (Karen-Mae Hong Son), klo-ue (Karen-Mae Hong Son), khi ka (Northeastern), <b>tumka khao</b> (Central), plu-wiat (Khmer), ma ting (Northern), ma ting ton (Northern), ma ting mak (Northern)
10	<i>Strychnos nux-vomica</i> L	kra chi (Central), ka kling (Central), kot ka kling (Central), tumka daeng (Central), saeng buea (Ubon Ratchathani), <b>salaeng chai</b> (Central), salaeng thom (Nakhon Ratchasima), salaeng buea (Nakhon Ratchasima), hong-buai-chi (Chinese)
11	<i>Strychnos polyantha</i> Pierre ex Dop	
12	<i>Strychnos rupicola</i> Pierre ex Dop	<b>khi ka khrua</b> (Prachin Buri)
13	<i>Strychnos thorelii</i> Pierre ex Dop	khiao ngu (Chumphon), chong la a (Chanthaburi), <b>chong ra a</b> (Chanthaburi), thao sa em (Trat), lum nok (Chumphon), <b>sa eng</b> (Trat)
14	<i>Strychnos vanprukii</i> Craib	<b>thao chang</b> (Northern)
15	<i>Strychnos villosa</i> A. W. Hill	-



**Botanical character**

*Strychnaceae* family is shrubs, trees, or lianas. If then lianas with axillary simple or double curled tendrils, sometimes with axillary thorns. Stipules often reduced to a straight ciliate ridge connecting petiole bases. Leaves petiolate to sometimes sessile; leaf blade margin entire, basal veins 3–7, secondary veins distinct 1–3 per side, from or near base and curved along margin. Inflorescences terminal and/or axillary, thyrsoid; bracts scalelike to sepal-like. Flowers pedicellate or sessile -4 or -5 merous. Corolla rotate to salverform; lobes valvate in bud, spreading to reflexed when open. Stamens inserted at corolla throat to middle of corolla tube, exerted to included; filaments long to short, mostly filiform; anthers orbicular to narrowly oblong, base mostly slightly -2 cleft, introrse, -2 locular and separate. Ovary 1 or -2locular; ovules few to many per locule. Style cylindrical; stigma capitate or faintly -2cleft. Berry orange or red when ripe in species represented, usually globose to ellipsoid, thin to thick walled, outside smooth to minutely warty, glabrous; pulp fleshy, usually orange; 1-15 seeded. Seeds ± flattened to saucer-shaped, circular to elliptic in outline; seed coat sericeous, felty, or scabrous and glabrous; embryo spatulate; endosperm horny; cotyledon leaflike [11].

## Plant description

### *Strychnos thorelii* Pierre ex Dop

*Lianas to 18 m. Twing thingly patently pubescent, glabrescent. Leaves oblong-ovate to lanceolate, 4.5-10 by 2-4 cm, chartaceous to coriaceous, shining above, sometimes sparsely patently hairy on the midrib beneath, otherwise glabrous; base broadly cuneate to subcordate, slighty attenuatr, apex gradually acute-acuminate; 3-5 plinerved above the base; petiole 0.5-1 cm, pubescent Inflorescences axillary and terminal, up to 10 cm long, lax, minutely pubescent, with some fruit only, branches slender, torus somewhat broadened. Fruits ovoid to oblong-ellipsoid, 2.5 by 1.5 cm, thin-shelled. Seed 1, elliptic-lenticular, 2 by 1.25 [12].*



**Figure 1** Leaves and stem of *Strychnos thorelii* Pierre ex Dop

*Strychnos lucida* R. Br.

Trees to 12 m tall, with spines when young. Branchlets sparsely pubescent or glabrous; branches grayish, rough with many small lenticels. Petiole 2–4 mm; leaf blade 2.5–10 × 1.5–6 cm, thin papery, glabrous, abaxially granular, base cuneate to slightly cordate, apex rounded, obtuse, or acute; basal veins 3–5. Thyrses terminal, ca. 9-flowered, pubescent. Calyx lobes broadly ovate. Corolla salverform, 1–1.5 cm; tube 7–12 mm, outside pubescent. Stamens inserted at corolla mouth, glabrous; filaments short; anthers oblong, 1.5–1.7 mm, apex exerted. Ovary globose, ca. 1 mm in diam. Style ca. 1.2 mm; stigma truncate. Berry globose, 2–2.5 cm in diam., smooth, glabrous, 2- or 3-seeded. Seeds shaped 1.2–1.5 × 1–1.2 cm [11].



Figure 2 Leaves, berry and stem of *Strychnos lucida* R.Br.

***Strychno nux-vomica* L.**

Trees to 25 m tall. Branchlets slightly pubescent, glabrescent. Petiole 0.5–1.5 cm; leaf blade suborbicular, broadly elliptic, or ovate, 5–18 × 4–12.5 cm, papery, abaxially minutely hairy especially on veins, adaxially glabrous and shiny, base rounded to cordate, apex short acuminate to acute and often mucronulate; basal veins 3–5. Thyrses axillary, 3–6 cm; peduncle puberulent; bracteoles pubescent. Flowers 5 merous. Pedicel puberulent, calyx lobes ovate, corolla greenish white to white, salverform, ca. 1.3 cm. Stamens inserted at mouth or corolla tube; filaments very short; anthers elliptic, ca. 1.7 mm, apex exerted. Pistil 1–1.2 cm. Ovary ovoid, glabrous. Style to 1.1 cm, glabrous; stigma capitate. Berry orange when ripe, globose, 2–4 cm in diam., glabrous, 1–4 seeded. Seeds orbicular to elliptic, 2–4 cm wide [11].



**Figure 3** Leaves, berries and seeds of *Strychnos nux-vomica* L.

***Strychnos nux-blanda* A. W. Hill**

*Trees to 15 m. leaves simple, opposite broadly ovate, 9-22 cm wide, 7-16 cm long. Inflorescence in axillary cymose panicle; flowers greenish yellow. The fruit are orange with shiny surface and they are globular, 5-8 cm in diameter, with a hard pericarp 2-3 mm thick, and contain up to 15 seeds resembling those of *S. nux-vomica* L. but tending to be more irregular in shape, slightly larger in size [11].*



**Figure 4** Leaves and seed of *Strychnos nux-blanda* A. W. Hill

**Medicinal uses**

This plant has been recognized as medicinal plant whose parts have been used as components in traditional medicine of various purposes [13]

Roots: antimalarial, cathartic and external use as anti-inflammatory for snake bite

Wood: relief of muscular pain and fevers

Leaves: external use as anti-inflammatory for swelling

Stem: topically apply for sprains and antiphlogistic for snake bite,

Bark: in high doses, used as a poison which stimulates a central nervous system that can cause violent muscular convulsions

## Chemical constituent of *Strychnos*

*Strychnos* plants are well-known as rich sources of bioactive indole alkaloids [14]. The Asian species are sources of strychnine and brucine, both of which obtained from seeds of *Strychnos ignatii* P. J. Bergius and *Strychnos nux-vomica* L. responsible for both the pharmacological and toxic properties, while the South American species are better known as the sources of certain types of curare [15].

### Indole alkaloids

Indole alkaloid is an aromatic heterocyclic organic compound [1]. It has a bicyclic structure, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrrole ring which can be substituted, either in oxidized or reduced forms, for example, *N*-acylindole, 2-acylindole, oxindole, pseudoindoxyl ( $\psi$ -indoxyl), indoline, *N*-acylindoline, methyleneindoline, indolenine, 7-hydroxy indolenine.

The indole alkaloids have been classified by their molecular skeletons into two types; simple indole alkaloids and complex or terpenoid indole alkaloids.

The simple indole alkaloids have uniformity structure. There are only indole nucleus (Figure 5) or derivatived indole nucleus, for example, harman and koenigine. Whereas the complex or terpenoid indole alkaloids are the derivation from a single precursor derived by the joining of an amino acid, tryptamine, a terpenoid and secologanin [16]. Chemical structure of harman, koenigine, tryptamine and secologanin were shown in Figure 6.

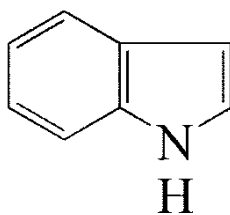


Figure 5 Chemical structure of Indole nucleus

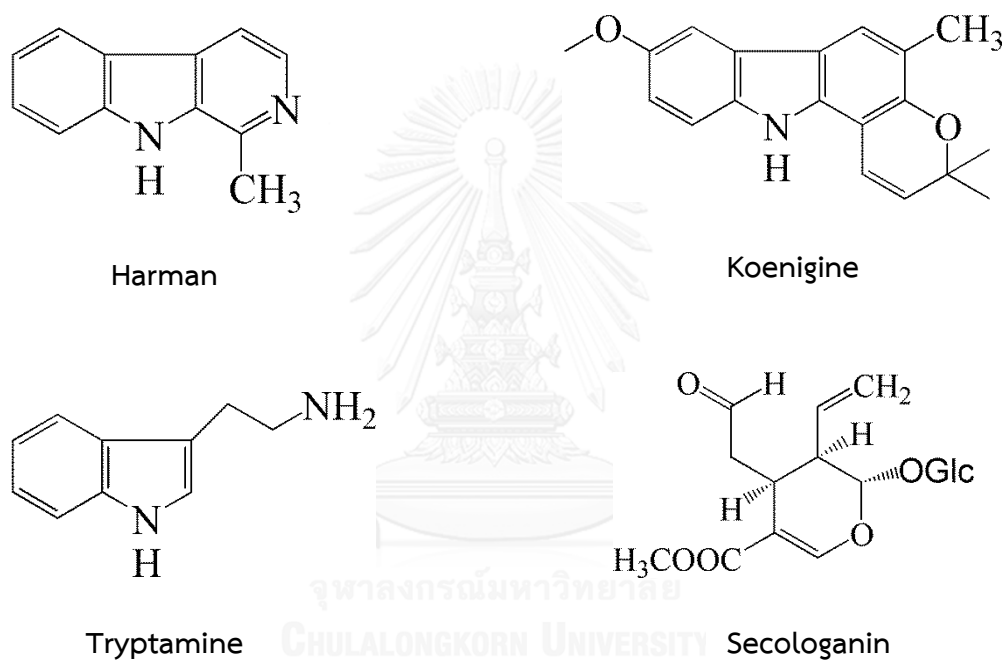


Figure 6 Chemical structure of harman, koenigine, tryptamine and secologanin

These complex indole alkaloids including monoterpenoid, bismonoterpenoid and trismonoterpenoid indole alkaloids found in *Strychnos* genus are summarized in Table 2.

**Table 2** Indole alkaloid from genus *Strychnos*

Plant	Plant part	Compounds	References
<i>S. nux-vomica</i>	Seed	<b><u>Monoindole alkaloids</u></b>	
		Strychnine	[3], [17]
		Brucine	[3], [18]
		$\beta$ -colubrine	[19]
		Pseudostrychnine	[11], [19]
		Pseudobrucine	[19]
		Strychnine <i>N</i> -oxide	[3], [18]
		Brucine <i>N</i> -oxide	[3],[18]
		16-hydroxy- $\alpha$ -colubrine	[19]
		2-hydroxy-3 methoxystrychnine	[19]
		Icajine	[11], [19]
		Vomicine	[19]
		Novacine	[19]
		Isostrychnine	[19]
		Isobrucine	[19]
Isostrychnine <i>N</i> -oxide	[19]		
Isobrucine <i>N</i> -oxide	[19]		
<i>S. icaja</i>	Root bark,	Strychnine	[20]
	Roots	Pseudostrychnine	[20]
		Protostrychnine	[20]
<i>S. henning</i>	Root bark	Diaboline	[11]
<i>S. variabilis</i>	Root bark	Retuline	[21]



Table 2 Indole alkaloid from genus *Strychnos* (Cont.)

Plant	Plant part	Compounds	References
<i>S. panganensis</i>	Root bark	<i>N</i> -desacetylisoretuline	[22]
		<i>N</i> -desacetylretuline	[22]
		12-hydroxy-11-methoxy- <i>N</i> -acetyl-nor-C-fluorocurarimine	[22]
		12-hydroxy-11-methoxy-nor-C-fluorocurarine	[22]
		<i>N</i> -desacetypermostrychnine	[22]
<i>S. myrtodies</i>	Stem bark	Strychnobrasiline	[23]
		Malagashanine	[23]
		12-hydroxymalagashanine	[23]
		Malagashanol	[23]
		Myrtoidine	[23]
		11-demethoxymyrtoidine	[23]
<i>S. diplotricha</i>	Stem bark	Myrtoidine	[23], [24]
		11-demethoxymyrtoidine	[24]
		3-epi-myrtoidine	[24]
<i>S. lucida</i>	Leaves	Brucine	[25]
		Pseudobrucine	[25]
		Brucine- <i>N</i> -oxide	[25]
		$\beta$ -colubrine	[25]
		Strychnine	[25]
		Pseudostrychnine	[25]

Table 2 Indole alkaloid from genus *Strychnos* (Cont.)

Plant	Plant part	Compounds	References
<i>S. usambarensis</i>	Root bark, Roots	<b><u>Bisindole alkaloids</u></b>	
		Usambarensine	[26], [27]
	Leaves	Dihydrousambarensine	[25],[26]
		10'-hydroxyusambarensine	[25]
		Methylusambarensine	[25]
		Usambarine	[25], [26]
		Dihydrousambarine	[25]
		11-hydroxyusambarine	[25]
		10-hydroxyusambarine	[25]
		Strychnopentamine	[25], [28]
		Isostrychnopentamine	[25], [27]
Chrysopentamine	[27]		
Longicaudatine	[26]		
<i>S. icaja</i>	Roots	Bisnordihydrotoxiferine	[29],[30]
		C-dihydrotoxiferine	[11]
		C-toxiferine	[11]
		Sungucine	[20], [28-29]
		Isosungucine	[25], [29]
		18-hydroxyisosungucine	[25]
		Strychnogucine A	[31]
		Strychnogucine B	[20], [30]
Strychnogucine C	[30]		

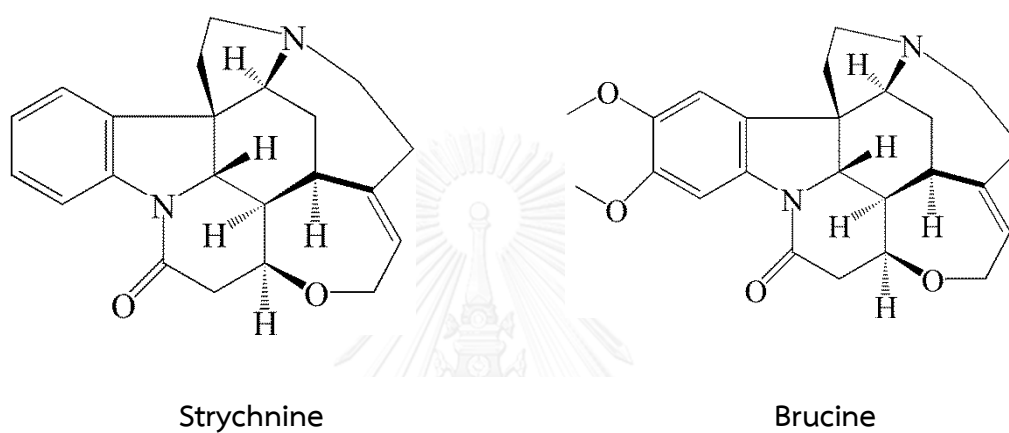
**Table 2** Indole alkaloid from genus *Strychnos* (Cont.)

Plant	Plant part	Compounds	References
<i>S. matopensis</i>	Roots	Matopensine	[26]
<i>S. kasengaensis</i>	Root bark	Matopensine <i>N</i> -oxide	[26]
<i>S. guianensis</i>	Stem bark	Guiaflavine	[32]
		5',6'-dehydroguiaflavine	[31]
		Guiachrysine	[33]
<i>S. icaja</i>	Roots	<b><u>Trisindol alkaloids</u></b>	[20],[29]
		Strychnohexamine	

Although the indole alkaloids are abundant in *Strychnos* plants, there have been reports that these plants also contain other compounds such as terpenoids and lignan glucosides (in stem of *S. vanprukii*) [34]. Phenolic and compounds and glucosides isolated from bark and wood of *S. axillari* [35]. In addition, iridoid glucosides are also found in seeds of *S. nux-vomica* L. too and quinic acid in in bark and wood of *S. lucida* R. Br. [36-37].

## Strychnine and brucine

Strychnine ( $C_{21}H_{22}N_2O_2$ , MW 334:41) is an indolomonoterpenic alkaloid possessing the strychnan group, which was isolated for the first time in 1818–1819 by Pelletier and Caventou [38] with brucine, its dimethoxylated analog. The chemical structure of strychnine and brucine were shown in Figure 7.



**Figure 7** Chemical structures of strychnine and brucine

Strychnine, the most abundant alkaloid of *S. nux vomica* L., is highly toxic to humans and most domestic animals. Strychnine is a well-known potent antagonist of glycine receptors in the vertebrate central nervous system and a strong blocker of various types of muscle and neuronal nicotinic acetylcholine receptors [39, 40]. Its poisoning is characterized by a short prodromal phase, after which there is an unusual combination of seizures with intact sensorium. Complications consist of hyperthermia, renal failure and rhabdomyolysis. The usual lethal dose of strychnine is reported to be between 50 and 100 mg, and the common cause of death is respiratory failure [41]. Brucine poisoning is rare, since it is usually ingested with strychnine, and strychnine is more toxic than brucine.

## Pharmacological investigations of *Strychnos* genus

### Antimalarial activities

The antimalarial activities of *Strychnos* alkaloids were further investigated in 2002 by Frederich and co-workers [27]. Sixty-nine alkaloids from various *Strychnos* species were subjected to *in vitro* antiplasmodial activities against chloroquine-resistant and chloroquine-sensitive lines of *Plasmodium falciparum*. The compounds, comprising mainly indolomonoterpenoid alkaloids, exhibited a wide range of biological potencies in the antiplasmodial assays. The most active alkaloids were also tested for cytotoxicity against HCT-116 colon cancer cells to determine their antiplasmodial selectivity. As a result of these studies, the alkaloids representing four types of bisindole skeleton exhibited potent and selective activities against plasmodium.

Philippe and co-workers [31] isolated a bisindole alkaloid, named strychnogucine C, and the first naturally occurring trimeric indolomonoterpenic alkaloid: strychnohexamine from the roots of *Strychnos icaja*. The *in vitro* antiplasmodial activities of these alkaloids have been determined against the FCA chloroquine-sensitive strain of *Plasmodium falciparum*. It was found that strychnogucine C possessed a weak activity ( $IC_{50} 16.1 \pm 0.76 \mu M$ ), which was notably less active than other sungucine type alkaloids: strychnogucine A ( $IC_{50} 2.3 \pm 0.30 \mu M$ ) and strychnogucine B ( $IC_{50} 0.6 \pm 0.07 \mu M$ ). On the other hand, strychnohexamine presented a strong antiplasmodial activity with an  $IC_{50}$  of  $1.1 \pm 0.10 \mu M$ , which was about two times more potent than bisnordihydrotoxiferine ( $IC_{50} 2.8 \pm 1.1 \mu M$ ).

### Antagonists of neuromuscular transmission activities

Wins and co-workers [42] presented the effective antagonists of nicotinic acetylcholine receptors in cultured human TE671 cells of constituents from the stem

bark of *Strychnos guianensis*. It was found that the most effective antagonist, guiachrysin, had an  $IC_{50}$  of 0.43  $\mu$ M whereas another bisindole alkaloid, guiaflavine was slightly less effective ( $IC_{50}$  0.70  $\mu$ M). Moreover, monoindole compounds were 10 to 100 times less potent than bisindole alkaloids.

### **Analgesic and anti-inflammatory activities**

Brucine and brucine N-oxide from seeds of *Strychnos nux-vomica* were reported that they possessed analgesic and anti-inflammatory activities. Both compounds significantly inhibited the released of prostaglandin  $E_2$  in inflammatory tissue, reduced acetic acid-induced vascular permeability and the content of 6-keto- $PGF_{1a}$  in Freund's complete adjuvant (FCA) induced arthritis rat's blood plasma [4].

### **Cytotoxic activities**

The cytotoxic activity of two bisindolomonoterpenic alkaloids, viz. sungucine isolated from the roots of *Strychnos icaja* and isostrychnopentamine from the leaves and root bark of *Strychnos usambarensis* were studied. Isostrychnopentamine was found to induce apoptosis in HCT-116 colon cancer cells by classical pathways and sungucine was able to induce apoptosis in HL-60 leukemia cells. This has been observed by several apoptosis tests: morphology, induction of caspase 3, cleavage of RARP, and fragmentation of DNA [43].

## Plant identification

The first step to categorize the herbal plant materials is the determination according to their macroscopic and microscopic characteristics for establishing the identity of herbal plant materials. Visual by eye based on the appearance of morphological characteristic provides the simplest and quickest inspection. However, macroscopic examination is sometime inadequate. It is often necessary to combine with other methods such as microscopic, chemical constituent or molecular analysis.

Previous studies refer to use of macroscopic and microscopic observation of the leaf morphological and anatomical characters for the *Strychnos* species identification and microscopic technique for comparative pharmacognostic studies and phytochemical studies on *Strychnos* specie [44-45].

## Macroscopic and microscopic evaluation

The macroscopic study of medicinal plants was helpful in rapid identification of plant material and also played an important role in standardization of drugs. The fresh leaves were subject to macroscopic evaluation for the morphological characters such as size, shape, color etc.

Microscopic examination focused on anatomical structures of plant materials such as the arrangement of stomata in epidermis or the presence/absence of compounds by using microscope.

### **Transverses section of midrib**

The qualitative microscopic evaluation of the transverse section of midribs and main veins demonstrated vascular tissues and particular surface cytomorphological characters such as trichomes, palisade cells, stomata, etc. Each cell type, form, size and its distribution within midrib cross section can provide distinguished identity for plant authentication. Moreover, midrib anatomical character enables to detect the contamination or adulteration in plant materials as well [46] .

### **Photomicroscope**

Microscopic evaluation use a digital camera attached above the microscope. It is more convenient than camera lucida. Anyhow, the specimen has to be very thin and the optical part used should be of very high quality as any defects accentuate in the final print. The photograph is recorded by digital camera attached above the microscope by helping of the scale labeling program. The photomicrography is uniquely qualified to be used for routine and advanced microscopic investigation of medicinal plant materials [46].

### **Quantitative microscopy**

In transverse sections it is not possible to study nature of epidermal cells, trichomes and stomata; stomatal index, vein islet number and veinlet termination number which play an important role in identifying characteristics of crude drugs and adulterants. However, these can be determined by quantitative microscopy. These quantitative microscopic values are comparatively constant for a particular species and can be used to make difference in closely related species.

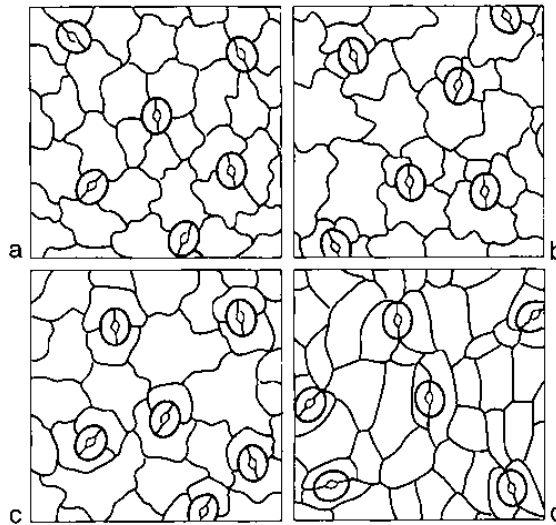


## Leaf measurement

### The stomatal number and the stomatal index

The stomatal number and the stomatal index is very specific criteria for identification and characterization of crude drugs. Four different types of stoma are often available for matured leaves that are distinguished by there from and arrangement in the surrounding cells. Type of stoma were revealed in Figure 8.

1. The anomocytic (irregular-celled) types: the stoma is surrounded by varying number of cells, which generally not different from those of the epidermis.
2. The anisocytic or cruciferous (unequal-celled) type: the stoma is usually surrounded by three or four subsidiary cells which one is markedly smaller than the other.
3. The diacytic or caryophyllaceous (cross-celled) type: the stoma is accompanied by two subsidiary cells, the common wall of which is at right angle to the stoma.
4. The paracytic or rubiaceous (parallel-celled) type: the stoma has two subsidiary cells with the parallel to long axis of the stoma.



**Figure 8** Types of leaf stomata in ventral surface area: a = Anomocytic type  
b = Anisocytic type, c = Diacytic type d = Paracytic type

### Palisade ratio

Palisade cells are a type of photosynthetic cells of the mesophyll of leaf occurring mostly just beneath the upper epidermal surface layer. Palisade ratio can be defined as the average number of palisade cells present beneath each upper epidermal cell. This value remains constant within a range for a given plant species and is of diagnostic value in differentiating the species. This value does not alter by geographical variation and differ from species to species [46]. It is a very useful diagnostic feature for characterization and identification of different plant species.

### Veinlet termination number

Veinlet termination number is defined as the number of veinlet termination per sq. mm of the leaf surface, midway between midrib of the leaf and its margin. It can be used as distinguishing character for the leaf of the same species or different species [47].

### **Epidermal cells area**

The epidermal cell area is defined as the surface area of epidermal cells per sq.µm of leaf.

### **Clarification reagents for microscopic analysis**

The presence of various contents within the cell such as starch grain, plastids, fat and oils etc., may give non-translucent section and obscure certain characteristics. There are some reagents that can dissolve of these contents and have been used to make a penetrating effect. Those sections may be more transparent and reveals details of the structures. Some of the reagents that most frequently used such as chloral hydrate and sodium hypochlorite are described below [47].

#### **Chloral hydrate solution**

Chloral hydrate is colorless hygroscopic crystal with melting point at 55°C. It's valuable and widely used as the best for clearing reagent. This solution dissolves starch, proteins, chlorophyll, resins, and volatile oils with the help of gentle warming. It does not dissolve calcium oxalate and causes the shrunken cells to expand without damage of cell wall or other tissue. Chloral hydrate is not only used for cross section, but also for whole leaves, flowers. [48]

#### **Sodium hypochlorite solution**

This solution is useful bleaching agent to remove deeply coloured sections such as many barks as well as for removing chlorophyll from the leaves [7]. The sections are immersed in the solution and left for a few minutes or until bleaching. The section should be removed from the solution and then washed with water when bleaching is completed.

## **Molecular evaluation**

The molecular method or DNA-based techniques have been widely used for herbal medicine technology and authentication of medicinal plant species. Some closely related species not easily characterized by general microscopy. Therefore, molecular methods are the most technique used for identification. These methods were useful in case of those that are frequently substituted or adulterated with other species. These techniques have been found to be useful and accurate for determination of genetic variation in plants [49].

DNA methods are suitable for identifying medicinal materials because genetic composition is unique for each individual irrespective of the physical forms of samples and are less affected by age, physiological conditions, environmental factors, harvest, storage and processing. Currently, sequence comparison or restriction analysis of fragments amplified with universal primers for organelle DNA has been widely used in species identification, genetic diversity and phylogenetic studies in many different plant species.

## **DNA isolation methods**

DNA extraction is a routine step in many biological studies including molecular identification. In addition, DNA extraction is often used in medical examinations, clinical diagnostics, and forensic investigations. Therefore, a variety of methods have been established to isolate DNA molecules from biological materials [50]. Recently, many DNA extraction kits are commercially available. Different extraction methods have various effects on DNA quantity and quality. An ideal extraction technique should be optimized to obtain maximize DNA yield, minimize DNA degradation, and be efficient in terms of cost, time, labor, and supplies. It must also be suitable for extracting multiple samples and generate minimal hazardous waste [51].

The extraction of the nucleic acids is difficult in a variety of plants because of the presence of polyphenols and secondary metabolites that interfere with DNA isolation procedures and downstream reactions such as DNA restriction, amplification and cloning [52]. A large number of secondary metabolites such as tannins, alkaloids, phenolics and terpenes responsible for the valuable pharmacokinetic properties of medicinal plants which interfere with the isolation process, tend to co-purify with DNA and interact irreversibly with proteins and nucleic acids [53]. Examples, problems encountered in the isolation and purification of high molecular weight DNA from certain legume plant species include: degradation of DNA due to endonucleases, co-isolation of highly viscous polysaccharides and inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with subsequent enzymatic reactions [54]. The separation of DNA from cellular components can be divided into four stages: 1. Disruption, 2. Lysis, 3. Removal of proteins and contaminants and 4. Recovery of DNA. In some methods, stage 1 and 2 are combined [55]. In general, all methods involve disruption and lysis of the starting material followed by the removal of proteins and other contaminants and finally recovery of the DNA.

### **CTAB method**

Doyle and Doyle have been used cetyltrimethyl ammoniumbromide (CTAB) to isolate DNA with the reducing agent  $\beta$ -mercaptoethanol in addition to proteinase K which removes protein [56]. CTAB is a cationic detergent, which solubilises membranes and forms a complex with DNA. Polyvinylpyrrolidone (PVP) has been also been used successfully to remove polyphenols along with a high molar concentration of NaCl to inhibit co-precipitation of polysaccharides and DNA. Most of the protocols recommend isolation of DNA from fresh tissues, but sometimes the samples collected from remote and rare locations may consist of plant parts in dry or semi-dry conditions [57]. Edwards

*et al.* have used Sodium dodecyl sulfate (SDS) and phenol instead of CTAB as a detergent for the same function of pure DNA isolation [58]. In the DNA preparation, it breaks up the lipids in the membranes to free the DNA from the cell.

### **DNA extraction kit**

Some researcher use a commercial kit based methods to supplement CTAB based extractions to generate genomic DNA of high enough quality to pass stringent conditions for library preparation [59]. Kit based extraction methods are intended to easily remove contaminants, but are often expensive, particularly when many samples are required for analysis. For example, the problem of losing DNA through subsequent column washes or precipitations can be exacerbated when only small amount of leaf tissue is available for collection. Commercial column base extraction kits, such as DNeasy® (Qiagen, USA) or Wizard® (Promega, USA), a spin-column of DNA-binding membrane together with a buffer system for cell lysis, DNA binding and elution were used in DNA extraction procedure.

### **Determination of genomic DNA quantity and purity**

DNA yield and purity were determined by two methods: agarose gel electrophoresis and spectrophotometer analysis. The yield was further measured by checking the optical density (OD) in a UV spectrophotometer at 260 nm. DNA purity determined by calculating the absorbance ratio at  $A_{260}/A_{280}$  for pure DNA is approximately 1.8. Agarose gel electrophoresis is a method to separate DNA or RNA molecular by size. This is achieved by moving negatively charge nucleic acid molecular though an agarose matrix with an electric field. Shorter molecule move faster and migrate faster than longer ones.

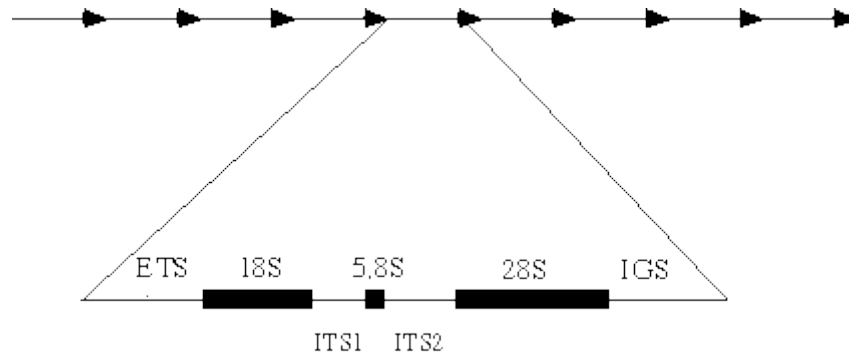
The obtained genomic DNA is then used as a DNA template for amplified the interested region. There are several regions in the DNA from various origins that used for studying the divergence or identity of plants such as;

### **Nuclear genome**

Nuclear genome is a linear DNA packed closely on the chromosome. It is the largest components in the nucleus. Nuclear genome is composed of information inherited equally from parents, one male, and one female. It is mostly used in forensic examinations. The regions of nuclear genome that commonly used in DNA fingerprint of herbal drug are;

### **Ribosomal DNA (rDNA)**

Ribosomal DNA codes for ribosomal RNA. The ribosome is a macromolecule in the cell that is able to produce proteins or polypeptide chains. rDNA consists of a tandem repeat of a unit segment which comprises of non transcribed spacer (NTS), external transcribed spacer (ETS), 18S, ITS1, 5.8S, ITS2, and 28S tracts (Figure 9). In the large rDNA array, polymorphisms between rDNA repeat units are very low which means low rate of polymorphism among species, indicating that rDNA tandem arrays are evolving through concerted evolution, so comparison of the rDNA segment including ITS region of the related species and phylogenetic analysis are accomplished [60].



**Figure 9** Diagram illustrating the organization of the internal transcribed spacers (ITS) region of the nuclear ribosomal DNA [61]

Internal transcribed spacers (ITS) are sequences located in eukaryotic ribosomal DNA (rDNA) genes between the 18S and 5.8S rDNA coding regions (ITS1) and between the 5.8S and 26S rDNA coding regions (ITS2) [62]. It has been found as parts of repeat units that are arranged in tandem arrays. The length and sequences of ITS regions of rDNA repeats are believed to be fast evolving. Universal PCR primers designed from highly conserved regions flanking the ITS and its relatively small size (600-700 base pairs) enable easy amplification of ITS region due to high copy of rDNA repeats. This makes the ITS region an interesting subject for evolutionary phylogenetic investigations [63]. The ITS region is typically been most useful for molecular systematics at the species level, and even within species

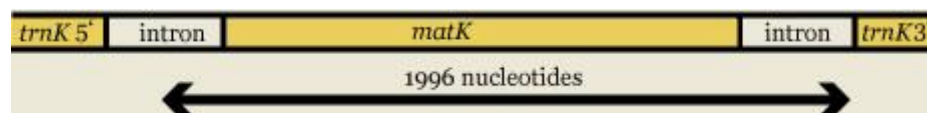
There are other regions in nuclear genome that are used in evolution analysis of plants but was not generally used in DNA fingerprint in herbal drug such as *phy* gene (phytochrome), *gapA* gene (glyceraldehydes-3-phosphate dehydrogenase), *adh* gene (alcohol dehydrogenase) and *pgi* gene (phosphoglucose isomerase).



### Chloroplast genome

Chloroplast genomes (cpDNA) are relatively large, usually approximately 140 kb in higher plants. Chloroplast genome codes for all the ribosomal RNA (rRNA) and transfer RNA (tRNA) species needed for protein synthesis. It has been used extensively to infer plant phylogenies at different taxonomic levels. Direct sequencing of polymerase chain reaction (PCR) products is now becoming a rapidly expanding area of plant systematics and evolution [64]. Chloroplast DNA is uniparental inheritance, so its pattern is homozygous which mean identical copies are present in the entire of a gene made sequencing easier. Chloroplast genome such as;

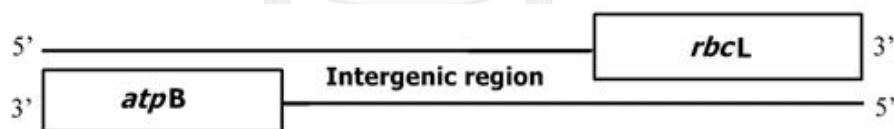
*matK* (Maturase K) gene is approximately 1500 base pairs in size, located within the intron of the chloroplast *trnK* gene (Figure 10). This gene can encode to enzyme maturase which presumably helps fold the intron RNA into the catalytically-active structure. The 3' end of the *matK* was identified to contain a conserved region of about 100 base/aminoacid. The *matK* gene is emerging as another valuable gene to study because of its reasonable size, high substitution rate, evenly distributed codon position variation, low transition and transversion ratio, and the easiness of amplification due to its two flanking coding *trnK* gene. The *matK* gene has fast evolution so, it is not possible to use the universal primer [65].



**Figure 10** Structure of *matK* gene which flanking between *trnK* gene regions [66]

The *rbcl* gene or RuBisCO (Ribulose-1,5-bisphosphate carboxylase/ oxygenase) is a single copy gene approximately 1430 base pairs in length. The *rbcl* gene is a gene coded for the large subunit of ribulose 1, 5 bisphosphate carboxylase/oxygenase and involved in catalyzing the primary chemical reaction by which inorganic carbon enters the biosphere which is first major step of carbon fixation. This gene has slow substitution rate and extensive database of sequences make *rbcl* sequence data well suited for phylogenetic studies at a variety of higher taxonomic levels, from interfamily to subclass [67].

*atpB* gene locates next to *rbcl* gene (Figure 11). Its common size is 1497 base pairs in plants. This gene encoded  $\beta$ -subunit of ATP synthase which is an enzyme catalyzes ATP synthesis. Their size, rate of evolution and lacking of intron, these are likewise to *rbcl* gene. Other chloroplast genomes are also used for investigating plants such as gene *ndhF*, the region in the area of gene *trnT*, *trnL* and *trnF*, etc. [68]



**Figure 11** Structure of *rbcl* gene and *atpB* gene

### Mitochondrial genome

Mitochondrial genome (mtDNA) is the DNA located in mitochondria which is involved in converting the chemical energy from food into adenosine triphosphate (ATP), an energy form that cells can use. Mitochondrial genome is large and vary in size, moreover, substitute rate of the nucleotide in plants mitochondrial genome is

slower than those of animals approximately 100-40 times and slower than those of nuclear genome and chloroplast genome around 12 and 4-3 times, respectively. Thus this genome is rarely used in authentication of herbal drugs [69].

### **Polymerase chain reaction (PCR)**

PCR is a scientific technique in molecular biology developed in 1983 by Kary Mullis. PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand. The PCR principle is to amplify a single or a few copies of a piece of DNA and generating thousands to millions of copies of a particular DNA sequence (Figure 12) [70]. A basic PCR set up requires several components and reagents such as, DNA template that contains the DNA region to be amplified, two primers that are complementary to the 3' ends of each of the sense and anti-sense strand of the DNA target, deoxynucleoside triphosphates (dNTPs; nucleotides containing triphosphate groups) which acts like the building-blocks from which the DNA polymerase synthesizes a new DNA strand, buffer solution, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase, divalent cations, magnesium or manganese ions; generally  $Mg^{2+}$  is used, but  $Mg^{2+}$  can be utilized for PCR-mediated DNA mutagenesis, as higher  $Mg^{2+}$  concentration increases the error rate during DNA synthesis [71], monovalent cation potassium ions and *Taq* polymerase or another DNA polymerase with a temperature optimum at around 70 °C. *Taq* DNA Polymerase is a highly thermostable DNA polymerase of the thermophilic bacterium *Thermus aquaticus*. The enzyme catalyzes 5' to 3' synthesis of DNA, it has no proofreading activity which is no detectable 3' to 5' exonuclease and possesses low 5' to 3' exonuclease activity. In addition, *Taq* DNA Polymerase exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Recombinant *Taq* DNA

Polymerase is ideal for standard PCR of templates 5 Kilo base (kb) or shorter. The error rate of *Taq* DNA Polymerase in PCR is  $2.2 \times 10^{-5}$  errors per nucleotide (nt) per cycle, as determined by a modified method that was described [72]. Accordingly, the accuracy of PCR is  $4.5 \times 10^4$ . Accuracy is an inverse of the error rate and shows an average number of correct nucleotides incorporated before an error occurs. The PCR is commonly carried out in a reaction volume of 10–100  $\mu$ l in small reaction tubes in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction [73]. A typical set of reactions might have a pre denaturation then, followed by 30-40 cycles of each comprising denaturation, annealing and extension. Then, evaluate the PCR product in 1.5% agarose gel electrophoresis which can separate nucleic acid molecules by size. Agarose gel that contains buffer is formed by a meshwork of molecules, and nucleic acids are driven through it by an electric field from charge negative to charge positive then visualize by staining the gel in ethidium bromide and observed under UV light[74]. There are some factors affect to the PCR exponential progression such as existing phenol or enzymes found in the sample which are inhibitors of the polymerase reaction, reagent limitation, accumulation of pyrophosphate molecules, and self-annealing of the accumulating product. The advantage of PCR can lead to many applications such as sequencing, genetic engineering, cloning, forensic biology, etc.

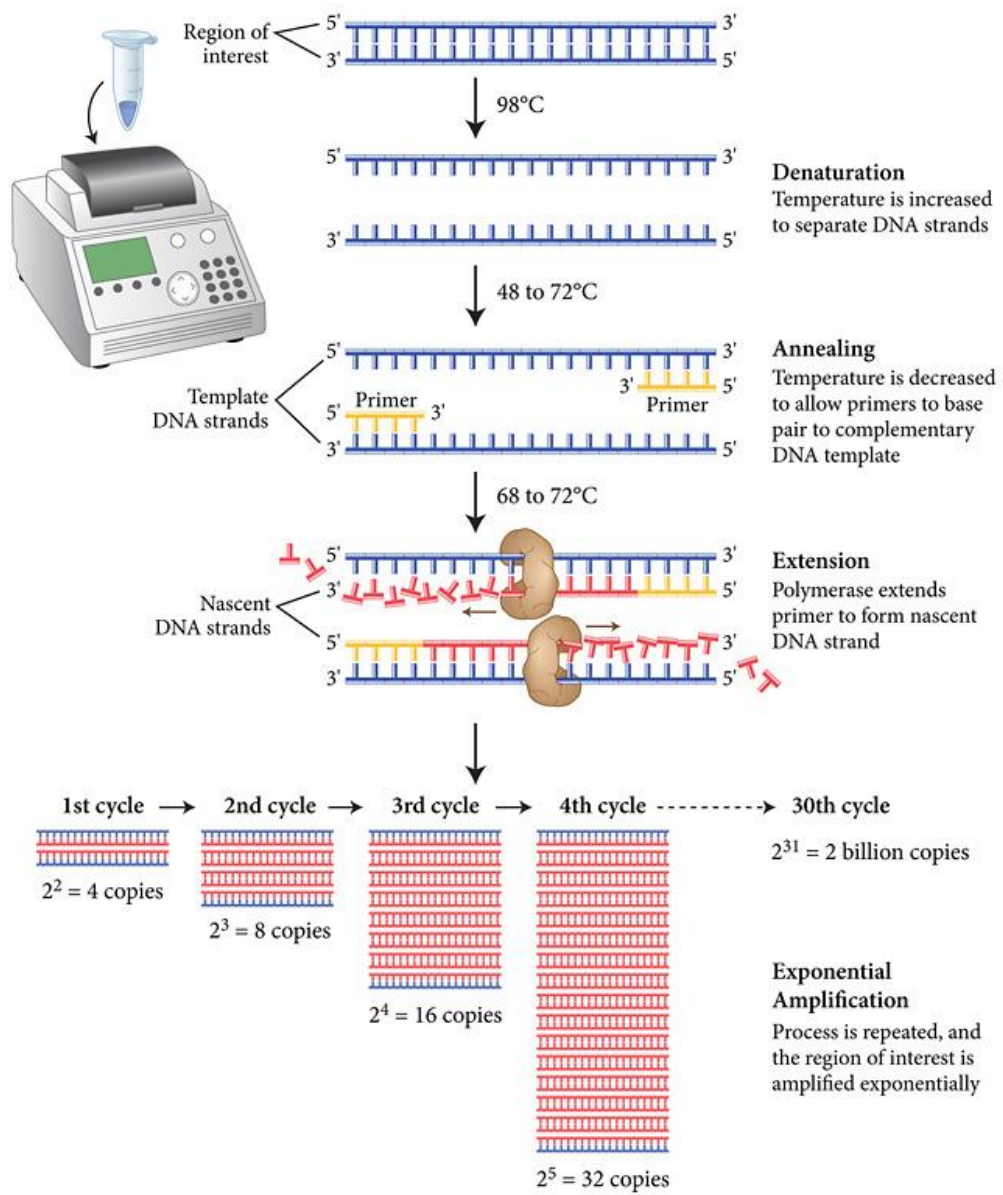


Figure 12 Illustration of the polymerase chain reaction (PCR) [75]

## DNA sequencing

DNA sequencing is used for determining the order of the nucleotides which are adenine, guanine, cytosine, and thymine in a molecule of DNA. Polymorphism at the DNA level can be studied by several methods but the direct strategy is determination of nucleotide sequences of a defined region. Nowadays, DNA sequencing is a routine technique in molecular biology laboratories.

Knowledge of DNA sequences has become indispensable for basic biological research, other research branches utilizing DNA sequencing, and in numerous applied fields such as diagnostic, biotechnology, forensic biology and biological systematics. The advent of DNA sequencing has significantly accelerated biological research and discovery [76]. The methods can be categorized as two major methods as;

### Maxam and Gilbert method

A sequencing method based on a chemical degradation was described by Maxam & Gilbert sequencing [77]. In this requires radioactive labeling at one 5' end of the DNA by a kinase reaction using gamma-<sup>32</sup>P ATP and purification of the DNA fragment. Chemical treatment generates breaks at a small proportion of one or two of the four nucleotide bases in each of four reactions (G, A+G, C, C+T) (Figure 13). For example, the purines (A+G) are depurinated using formic acid, the guanines (and to some extent the adenines) are methylated by dimethyl sulfate, and the pyrimidines (C+T) are methylated using hydrazine. The addition of salt (sodium chloride) to the hydrazine reaction inhibits the methylation of thymine for the C-only reaction. The modified DNAs are then cleaved by hot piperidine at the position of the modified base. The concentration of the modifying chemicals is controlled to introduce on average one modification per DNA molecule. Thus a series of labeled fragments is generated, from the radiolabeled end to the first "cut" site in each molecule. The fragments in

the four reactions are electrophoresed side by side in denaturing acrylamide gels for size separation. To visualize the fragments, the gel is exposed to X-ray film for autoradiography, yielding a series of dark bands each corresponding to a radiolabeled DNA fragment, from which the sequence may be inferred [78].

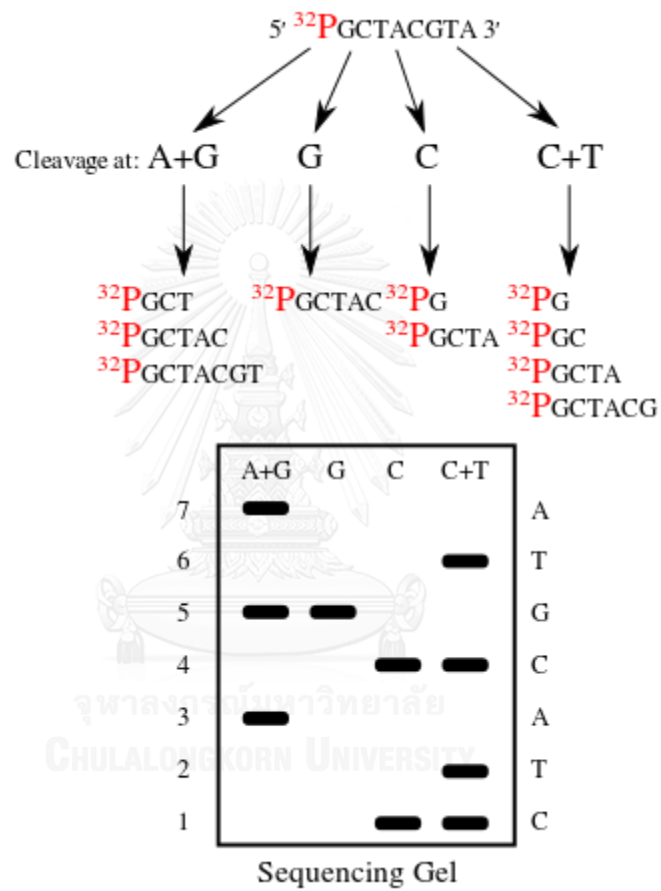


Figure 13 Maxam and Gilbert method [79]

### Sanger's method

Sanger sequencing is a method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during *in vitro* DNA replication. This method, developed by Frederick Sanger and colleagues in 1977, was initially known as the chain termination method or dideoxynucleotide method (Figure 14). This method is better than chemical method because of the lower of toxic chemicals and lower amount of radioactivity is used. The method requires a single-stranded DNA template, a DNA primer, a DNA polymerase, normal deoxynucleotidetriphosphates (dNTPs; dATP, dGTP, dCTP and dTTP), and modified nucleotides (dideoxynTPs; ddATP, ddGTP, ddCTP, or ddTTP), lacking a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides, thus terminating DNA strand extension and resulting in DNA fragments of varying length. These ddNTPs will also be radioactively or fluorescently labelled for detection in automated sequencing machines. The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotides. The newly synthesized and labelled DNA fragments are heat denatured, and separated by size on a denaturing polyacrylamide-urea gel electrophoresis with each of the four reactions run in individual lanes (lanes A, T, G, C); the DNA bands are then visualized by autoradiography or UV light, and the DNA sequence can be directly read off the X-ray film or gel image. X-ray film was exposed to the gel, and the dark bands correspond to DNA fragments of different lengths. A dark band in a lane indicates a DNA fragment that is the result of chain termination after incorporation of a dideoxynucleotide (ddATP, ddGTP, ddCTP, or ddTTP). The relative positions of the different bands among the four lanes are then used to read (from bottom to top) the DNA sequence.



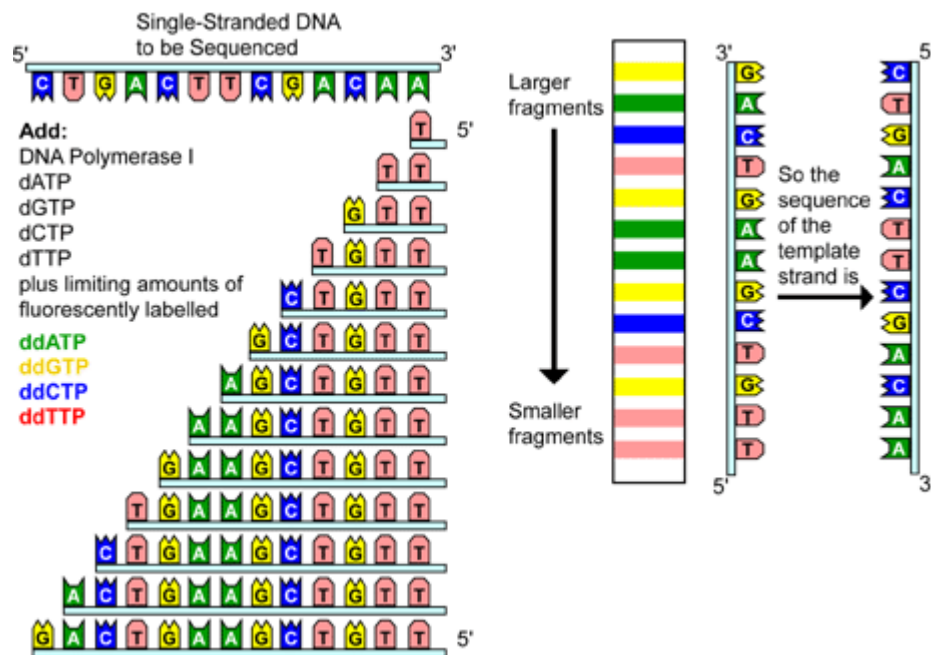


Figure 14 Sanger's method [80]

### PCR-RFLP analysis

PCR-restriction fragment length polymorphism (RFLP)-based analysis is a popular technique for genetic analysis. It has been applied for the detection of intraspecies as well as interspecies variation. There exist several techniques that are related with PCR-RFLP and also involve gel electrophoresis including techniques for DNA fingerprinting and expression profiling. The first step in a PCR-RFLP analysis is amplification of a fragment containing the variation. This is followed by treatment of the amplified fragment with an appropriate restriction enzyme. Since the presence or absence of the restriction enzyme recognition site results in the formation of restriction fragments of different sizes, allele identification can be done by electrophoretic resolution of the fragments. PCR-RFLP is an extremely valuable technique for genotyping of species-specific variations [81].

## CHAPTER III

### MATERIALS AND METHODS

#### Chemicals and Reagents

1. Ethanol (Merck, Germany)
2. Chloral hydrate (Ajax Finechem Pty Ltd., Australia)
3. sodium hypochlorite (Hiter Bleach, Kao industrial, Thailand)
4. CTAB: Hexadecyltrimethylammonium bromide (Sigma-Aldrich Company Co., St. Louis, MO, USA)
5. Liquid nitrogen
6.  $\beta$ -mercaptoethanol (Sigma-Aldrich Company Co., St. Louis, MO, USA)
7. Chloroform (Merck, Darmstadt, Germany)
8. Isoamyl alcohol (Sigma-Aldrich Company Co., St. Louis, MO, USA)
9. Absolute ethanol (Merck, Darmstadt, Germany)
10. Sodium acetate (BDH Laboratory supplies, Poole, England)
11. Tris (hydroxymethyl)-aminomethane hydrochloride (Fluka, Biochemika, Germany)
12. EDTA: Ethylenediaminetetraacetic acid (Merck, Darmstadt, Germany)
13. NaCl: Sodium hydroxide (BDH Laboratory supplies, Poole, England)
14. Agarose (Ultrapure TM, Life technologies, USA)
15. Ethidium bromine (Sigma-Aldrich Company Co., St. Louis, MO, USA)
16. 1 kb DNA Ladder (Promega, USA)
17. Loading Dye (bromophenol blue, Fermentas, USA)
18. *Taq* DNA polymerase (Fermentas, USA)
19. forward - reverse primer primer (Operon Biotechnologies, Germany)
20. 10X PCR Buffer (Fermentas, USA)
21. MgCl<sub>2</sub> (Fermentas, USA)

22. Distilled water

23. DNA template

### Materials

1. Microcentrifuge tube (Axygen, USA)
2. Pipet tips (Axygen, USA)
3. PCR tubes (Axygen, USA)
4. PCR purification kit (QIAGEN, USA)

### Instrumentations

1. Microphotographs were taken using digital camera (Canon Power shot A640, Japan) attached to the Photomicroscope (Zeiss Image A.2 Axio, Germany)
2. Micropipette (Eppendorf, Germany)
3. Mortar
4. UV transilluminator (AutoChem TM system, USA)
5. Thermal cycler (*Geneamp PCR 9700*, Applied Biosystems)
6. Gel electrophoresis apparatus and power supply
7. Centrifuge (*Biofuge Pico*, Kendro, Germany)
8. Vertex mixer
9. shaking incubator

## Methods

### Sample collection and authentication

The leaves of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* were collected from different locations in Thailand. They were authenticated by Associate Professor Dr. Nijsiri Ruangrunsi. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand.

### Macroscopic evaluation

Whole plant characters of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* were observed and recorded. The drawing outlines of whole plants were in proportion size which related to real size. Leaves morphological characteristic (leaves shape, base, texture, venations) were observed and recorded.

### Microscopic evaluation

Microscope evaluation use a digital camera attached above the microscope (Figure 15). The photograph is recorded with an attached digital camera Cannon Power shot A640 and examining under the photomicroscope Zeiss Image A.2, Axio with objective lens. The images were recorded using AxioVision Release 4.8.3 software. This software was used for images alignment and labeling. The individual of each sample was studied under eyepiece lens of 10X magnifications and objectives with a 5X 10X, 20X and 40X magnifications.



**Figure 15** The photomicroscope (Zeiss Image A.2 Axio, Germany) with an attached digital camera (Cannon Power shot A640, Japan)

## Methods for microscopic analysis

### Transverse section of plant

The fresh mature leaves were cleaned. Transverse section was prepared by cutting the leaves in parallel including the midrib and lamina into pieces as thin as possible and transferred these tissue sections by a brush moistened with water. Selected satisfactory sections were prepared and mounted onto a slide in glycerin for microscopic examination under photomicroscope, scaled for labeling size of each character. Transverse sections of midrib were drawn in the proportion size related to the original scale in drawing paper.

### Stomata classification of leaves

The fresh mature leaves were cleaned and cut into small pieces (1x1 cm), in the central part of lamina, midway between the midrib and the margin. Small pieces of leaves were soaked in sodium hypochlorite solution (Haitec bleaching solution: distilled water, 1:2) for 24 hour and cleared by gently warming with chloral hydrate

solution (chloral hydrate: distilled water, 9:1). The thin membranous layer which was sufficiently big enough for the field vision were taken on glass slide.

### **Leaf constant numbers**

The fresh mature leaves were cleaned and clear with chloral hydrate solution using the same procedure as described in part stomata classification of leaves. Determined a total of 30 fields for the stomatal number, stomatal index, palisade ratio, veinlet termination number epidermal cell number and epidermal cell area. That were recorded and presented by mean and standard deviation (SD). Then, mean and SD of each sample were averaged as the constant number of each species. The methods were carried out as below;

#### **a) Determination of stomatal number and stomatal index**

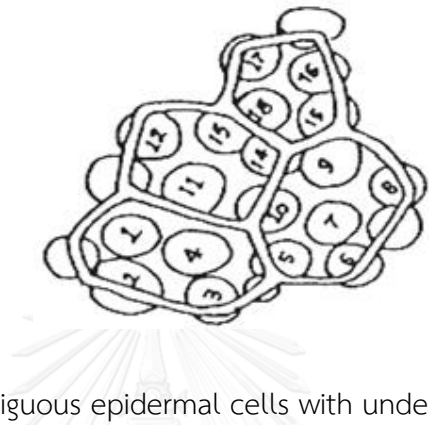
Stomatal number is the average number of stomata per square mm of epidermis and the number on each surface of a leaf. Stomatal index (SI) is one of the more distinguishing characteristic for herbal leafy drugs. Stomatal is defined as the percentage of stomata from the total number of epidermal cell, which can be explained as:

$$SI = \frac{S}{E+S} \times 100$$

Where S = the number of stomata in a given area of leaf; and E = the number of epidermal cells in the same area of leaf

### b) Determination of palisade ratio

Palisade ratio is the average number of palisade beneath one epidermal cell of a leaf. It is determined by counting the palisade cells beneath four continuous upper epidermal cell. The palisade cells in surface view were shown in Figure 16.



**Figure 16** Four upper contiguous epidermal cells with underlying palisade cells in surface view

### c) Determination of veinlet termination number

Small vascular bundle surrounded by many conducting tissue is call veinlet. The end termination of the vein is total number of veinlet termination point present per sq.mm on the surface of leaf.

### d) Determination of epidermal cell number and epidermal cell area

Epidermal cell number was calculated by counting the number of each field (1 sq.mm), epidermal cell area was reported per sq.µm

## Molecular analysis

### DNA extraction

#### *Preparation of CTAB buffer*

Genomic DNA was individually extracted from the fresh young specimen leaves using a modified CTAB method. The preparation of CTAB buffers (4  $\mu$ l of 2 mercaptoethanol was immediately added to each 1 ml of CTAB buffers before used) were shown in Table 3.

**Table 3** Preparation of CTAB buffers

Stock reagent	Final concentration	Final amount
CTAB	2% (w/v)	2 g
1 M Tris-HCL pH8	100 mM	10 ml
0.5 M EDTA	20 mM	4 ml
5 M NaCl	1.4 M	28 ml

Make up with distilled water to 100 ml

Add 4  $\mu$ l of 2 mercaptoethanol to each 1 ml of CTAB buffers before used.

### Procedure

For DNA extraction procedure, fresh leaves were ground with liquid nitrogen to fine powder in mortar. The ground powders were transferred into 1.5 ml microcentrifuge tube using spatula. Then 500  $\mu$ l of CTAB buffer, vortex and incubate the CTAB/plant mixture at 65 °C for an hour in shaking incubator. After incubation, centrifuge for 10 minutes to spin down cell debris. Transfer the supernatant to a new microcentrifuge tube and 500  $\mu$ l of chloroform was added. Vortex and then centrifuge



for 10 minutes. Transfer the aqueous phase (upper) to a new microcentrifuge tube, 500  $\mu$ l of chloroform/isoamyl alcohol (24:1) was added, vortex and then centrifuged for 10 minutes. Depending upon the purification of DNA, the aqueous phase may be re-extracted. Be carefully transferred the final upper aqueous phase to a clean microcentrifuge tube. Then 1:10 volume of 3M sodium acetate pH 5.0 was added followed by 2 volume of ice-cold (-20 °C) absolute ethanol, and mix by slowly invert the tube. Let the tube stand at -20 °C for an hour to precipitate DNA. After precipitation, centrifuge for 10 min and remove all of the supernatant. DNA was then washed with 1 ml of cold 70% ethanol and gently inverted the tube several times. Then centrifuge 10,000 rpm for 10 minutes and discard all the supernatant and allowed DNA pellet to dry at room temperature. Do not leave the DNA to over dry because it will be hard to re-dissolve. Dissolved DNA in 100  $\mu$ l TE buffer (10 mM Tris pH 8, 0.1 mM EDTA pH8) and stored at -20 °C further use as templates in PCR amplification.

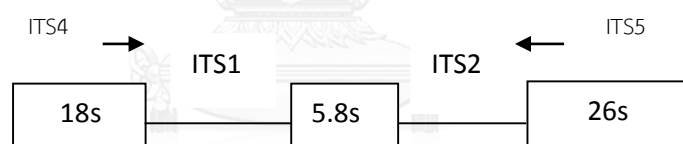
#### **Determination of genomic DNA quantity**

Five  $\mu$ l of genomic DNA were analyzed by 1.5% agarose gel electrophoresis and with 1 kb marker. Stained by ethidium bromide and visualized under UV illuminator.

## PCR amplification

### PCR amplification of ITS region

The ITS including ITS1 - 5.8S rDNA - ITS2 region was amplified using a pair of universal primer (ITS5 and ITS4) as shown in Figure 17 and Table 4. PCR amplification was performed in a 25  $\mu$ l reaction volume, containing of 1X PCR buffer (100 mM) KCl, 20 mM Tris-HCl (pH 8), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2  $\mu$ M of each primers, 0.5 unit of *Taq* DNA polymerase and 1  $\mu$ l of DNA template. PCR amplification was performed in Thermal cycler under the following condition: initial denaturation step at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 30 second, primer annealing at 55 °C for 30 second, primer extension at 72 °C for 30 second, a final extension at 72 °C following 5 minutes, and then hold at 4 °C. Five microliter of PCR product was separated by 1.5% agarose gels electrophoresis in 1XTBE buffer and stained with ethidium bromide.



**Figure 17** The organization of plant ribosomal RNA gene

**Table 4** The ITS primers used in this PCR amplification

Primer	Direction	Sequencing (5'-3')	Length (bp)	Tm (°C)
ITS5	Forward	GGAAGTAAAAGTCGTAACAAG G	22	55
ITS4	Reverse	TCCTCCGCTTATTGAGC	20	56

### PCR amplification of *rbcl* gene

*rbcl* gene was amplified using a pair of primer as shown in Figure 18 and Table 5. PCR amplification was performed in a 25  $\mu$ l reaction volume, containing of 1X PCR buffer (100 mM KCl, 20 mM Tris-HCl (pH 8.0), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2  $\mu$ M of each primers, 0.5 unit of *Taq* DNA polymerase and 1  $\mu$ l of DNA template. PCR amplification was performed in Thermal cycler under the following condition: initial denaturation step at 95  $^{\circ}$ C for 5 minutes, followed by 30 cycles of denaturation at 95  $^{\circ}$ C for 30 second, primer annealing at 55  $^{\circ}$ C for 30 second, primer extension at 72  $^{\circ}$ C for 30 second, and a final extension at 72  $^{\circ}$ C for 5 minutes, and then hold at 4  $^{\circ}$ C. Five microliter of PCR product was separated by 1.5% agarose gels electrophoresis in 1X TBE buffer and stained with ethidium bromide.



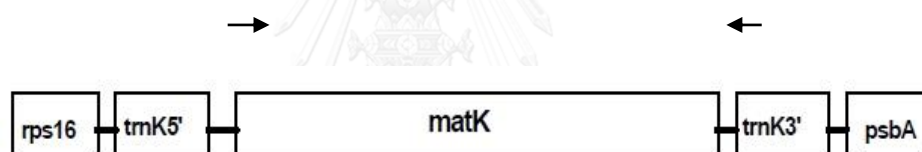
Figure 18 Structure of *rbcl* gene

Table 5 The *rbcl* primers used in this PCR amplification

Primer	Direction	Sequencing (5'-3')	Length (bp)	T <sub>m</sub> ( $^{\circ}$ C)
<i>rbcl</i>	Forward	TGTCACCACAAACAGAGACTAAAGCA	29	62
<i>rbcl</i>	Reverse	AGTCTTTTAGTAAAGATTGGGCCGAG	23	59

### PCR amplification of *matK* gene

*matK* gene was amplified using a pair of primers as shown in Figure 19 and Table 6. PCR amplification was performed in a 25  $\mu$ l reaction volume, containing of 1X PCR buffer (100 mM KCl, 20 mM Tris-HCl (pH 8.0), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.2  $\mu$ M of each primers, 0.5 unit of *Taq* DNA polymerase and 1  $\mu$ l of DNA template. PCR amplification was performed in Thermal cycler under the following condition: initial denaturation step at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 30 second, primer annealing at 54 °C for 30 second, primer extension at 72 °C for 1 minute, a final extension at 72 °C following 5 minutes, and then hold at 4 °C. . Five microliter of PCR product was separated by 1.5% agarose gels electrophoresis in 1X TBE buffer and stained with ethidium bromide.



**Figure 19** Structure of *matK* gene and the position of primer

**Table 6** The *matK* primers used in this PCR amplification

Primer	Direction	Sequencing (5'-3')	Length (bp)	T <sub>m</sub> (°C)
<i>trnK</i>	Forward	CTGTTGATAAGTTTACCTGCCTCCG	25	70
<i>trnK</i>	Reverse	ATTGCACACGGCTTTCCTATG	22	66

### Preparation of 1.5 %agarose gel

Weight 1.5 g of agarose in 100 ml of 1X TBE buffer and solubilized by heating in microwave. Then, let the gel solution to warm before pouring into a plastic tray. After the gel was solid, removed the comb and put the tray into a gel electrophoresis apparatus fulfilled with 1X TBE buffer in chamber. Five microliter of each amplified PCR products was analyzed in 1.5% agarose gel electrophoresis comparison with 1 kb molecular weight marker. Electrophoresis was performed at constant voltage of 100 volts until the faster migration dye (bromophenol blue) has traveled at the end of gel and then stained with ethidium bromide. The agarose gel was visualized under UV transilluminator and photographed.

### DNA sequencing analysis

#### Procedure

The PCR products were purified by PCR purification kit (QIAGEN) according to the manufacturer's protocol prior sequencing (ABI system). The ITS region, *rbcL* and *matK* sequences from both sense and antisense stand were sequenced and analyzed using BioEdit sequence alignment version 7.0.9 for Windows.

## PCR-RFLP method

The *matK* sequences data were analyzed and the PCR products were digested with restriction enzymes (*DraI*, *XbaI*) according to manufacturer's instructions. Restriction endonucleases recognition site of *DraI* and *XbaI* were shown in Table 7.

**Table 7** List of restriction endonucleases in PCR-RFLP method

Restriction enzyme	Genomic source	Sequencing	Restriction condition
<i>DraI</i>	<i>Deinococcus radiophilus</i>	5'... TTT <sup>▼</sup> AAA...3' 3'... AAA <sup>▲</sup> TTT...5'	37 °C
<i>XbaI</i>	<i>Xanthomonas badrii</i>	5'... TCTAGA...3' 3'... AGATC <sup>▲</sup> T...5'	37 °C

## Procedure

The digestion of PCR products was performed according to instructions from the manufacturer. The reaction mixture was carried out in 20 µl which consisting of 10 µl of *matK* gene PCR amplification product, 2 µl of restriction buffer, 10 U/µl restriction enzyme. The reaction was incubated at temperature 37 °C for 30 minute in shaking incubator. Ten microliter of the restriction reaction were separated through their length by 1% agarose gel electrophoresis along with 1 kb DNA ladder. Electrophoresis was performed in 1X TBE buffer at constant voltage of 80 volts until the faster migration dye (bromophenol blue) has traveled to two-third of gel and then stained with ethidium bromide. Fragment patterns were analyzed under UV transilluminator and photographed.

## CHAPTER IV

### RESULTS

#### Sample collection

Total twelve specimens of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* were collected from Bangkok, Chachoengsao, Songkla, Chonburi, Nonthaburi, Chiangmai and Pathum Thani provinces in Thailand, during August, 2014 to February, 2015. The collecting locality details were shown in Table 8.

**Table 8** Leaves of selected *Strychnos* species from difference locations in Thailand

<i>Strychnos</i> Species	Sample no	Location	Collecting date	Voucher ID
<i>S. thorelii</i>	1	Chachoengsao	Aug, 2014	St1
	2	Chachoengsao	Aug, 2014	St2
	3	Chonburi	Oct, 2014	St3
<i>S. lucida</i>	1	Bangkok	Sep,2014	Sl1
	2	Pathum Thani	Sep,2014	Sl2
	3	Nonthaburi	Feb, 2015	Sl3
<i>S. nux-vomica</i>	1	Pathum Thani	Sep, 2014	Sv1
	2	Khon Kaen	Nov, 2014	Sv2
	3	Songkhla	Nov, 2014	Sv3
<i>S. nux- blanda</i>	1	Chiang Mai	Nov, 2014	Sb1
	2	Chiang Mai	Nov, 2014	Sb2
	3	Chiang Mai	Nov, 2014	Sb3

## Macroscopic and microscopic evaluations

### Macroscopic evaluation

Macroscopic evaluation of four *Strychnos* species were revealed as followed;

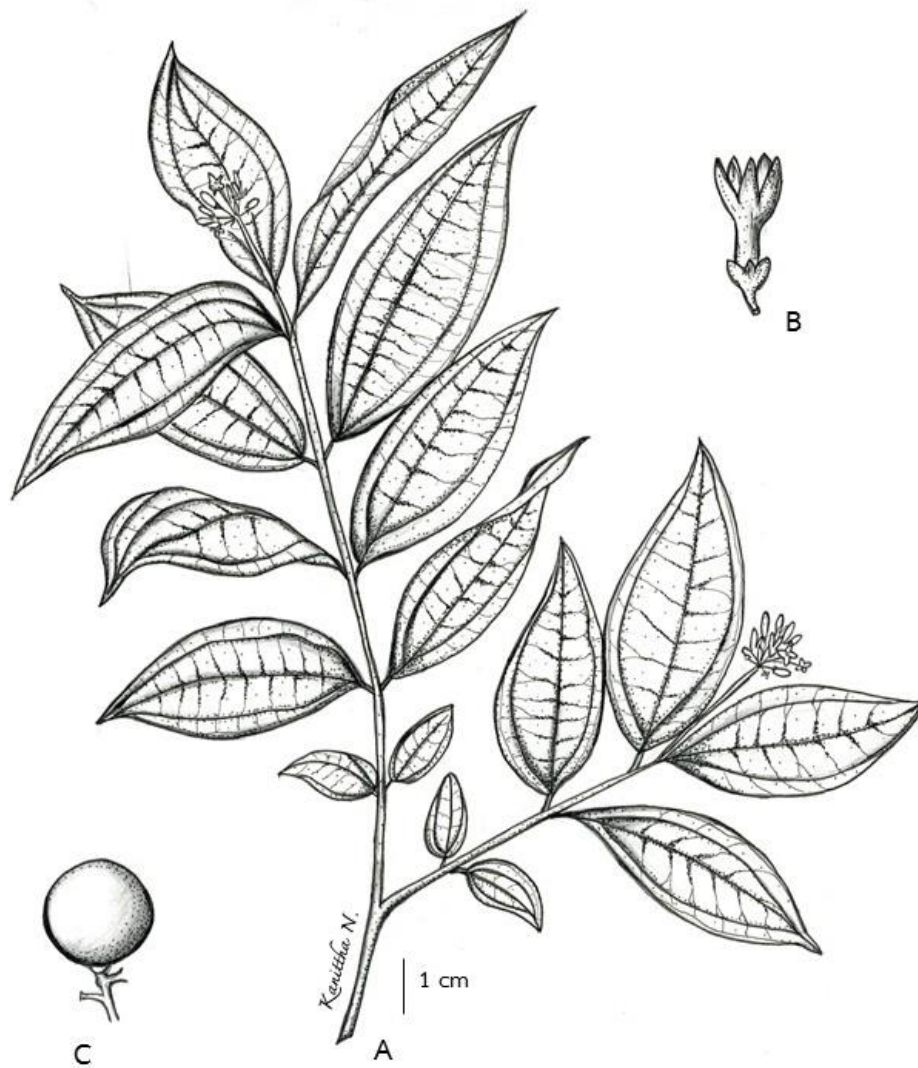
#### *Strychnos thorelii* Pierre ex Dop

Leaves oblong-ovate to lanceolate, 4.5 to 14 long, 2 to 5 cm wide, chartaceous to coriaceous, shining above, sometimes sparsely patently hairy on the midrib beneath, otherwise glabrous; base broadly cuneate to cordate, apex gradually acute-acuminate, , basal veins 3-5. Leaves of *S. thorelii* were shown in Figure 20. The whole plant of *S. thorelii* was demonstrated in Figure 21.



Figure 20 Leaves of *Strychnos thorelii* Pierre ex Dop





**Figure 21** The whole plant of *Strychnos thorelii* Pierre ex Dop (A)  
flower (B) and berry (C)

*Strychnos lucida* R.Br.

Leaf blade 2.5 to 10 long, 1.5 to 6 cm wide, thin papery, glabrous, abaxially granular, base cuneate to cordate, apex rounded, obtuse, or acute, basal veins 3-5. Leaves of *S. lucida* in Figure 22. The whole plant of *S. lucida* was demonstrated in Figure 23.



Figure 22 Leaves of *Strychnos lucida* R.Br.

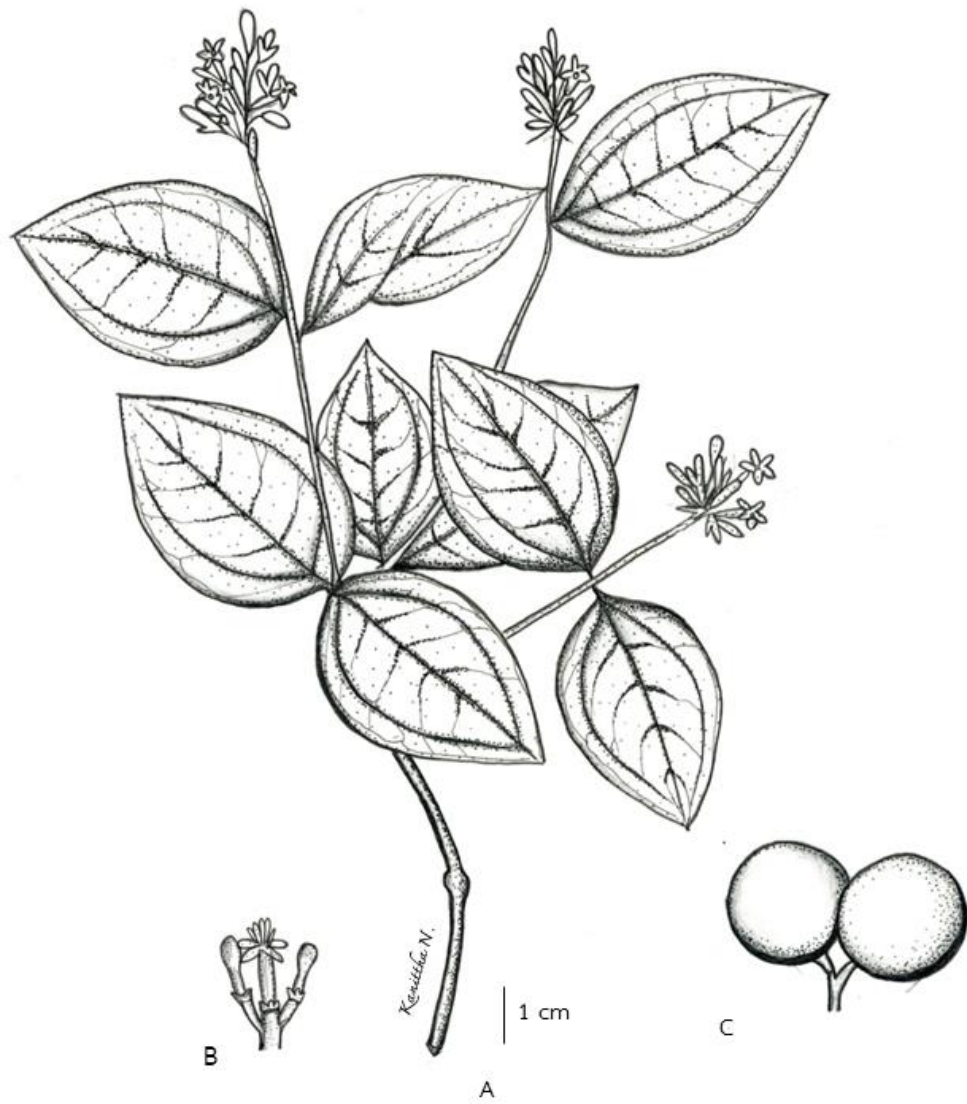


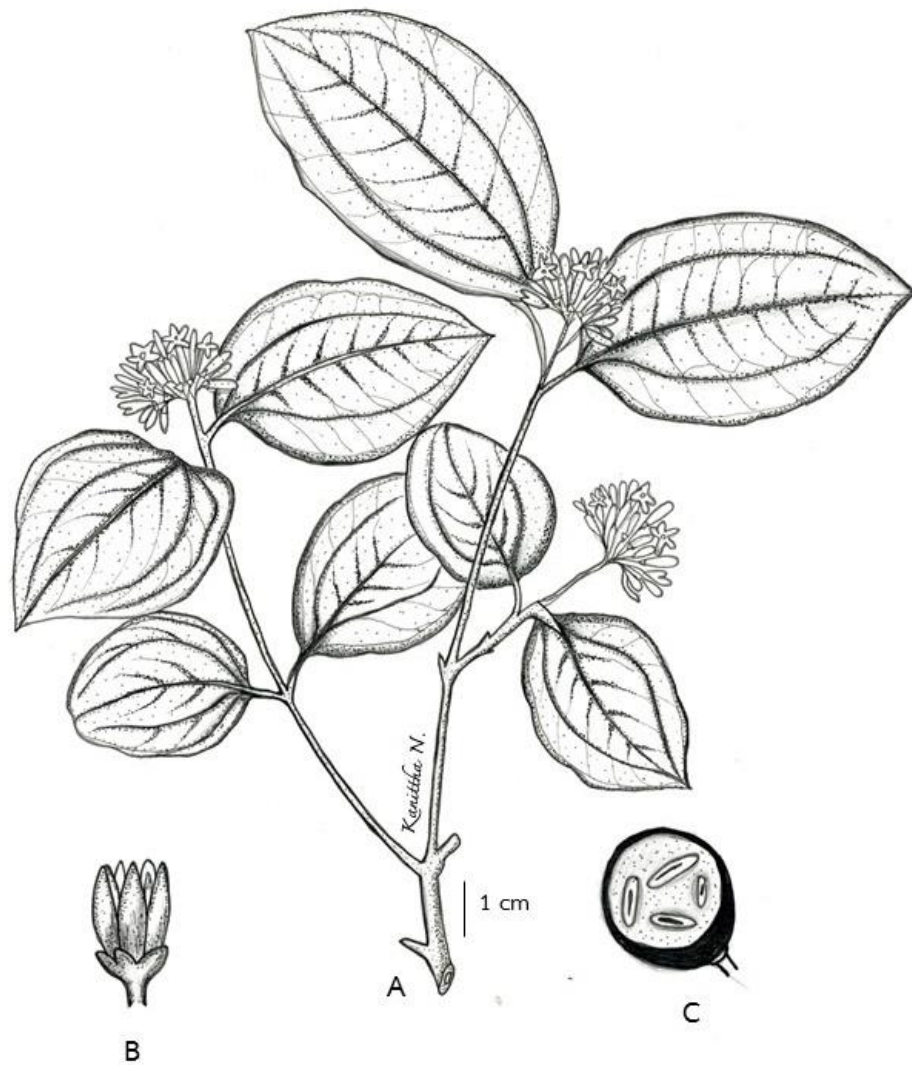
Figure 23 The whole plant of *Strychnos lucida* R.Br.( A),  
flower (B) and berries (C)

*Strychnos nux-vomica* L.

Leaf blade suborbicular, broadly elliptic, or ovate, 5 to 18 long, 4 to 12.5 cm wide, papery, abaxially minutely hairy especially on veins, adaxially glabrous and shiny, base rounded to cordate, apex short acuminate to acute and often mucronulate, basal veins 3-5. Leaves of *S. nux-vomica* were shown in Figure 24. The whole plant of *S. nux-vomica* was demonstrated in Figure 25.



**Figure 24** Leaves of *Strychnos nux-vomica* L.



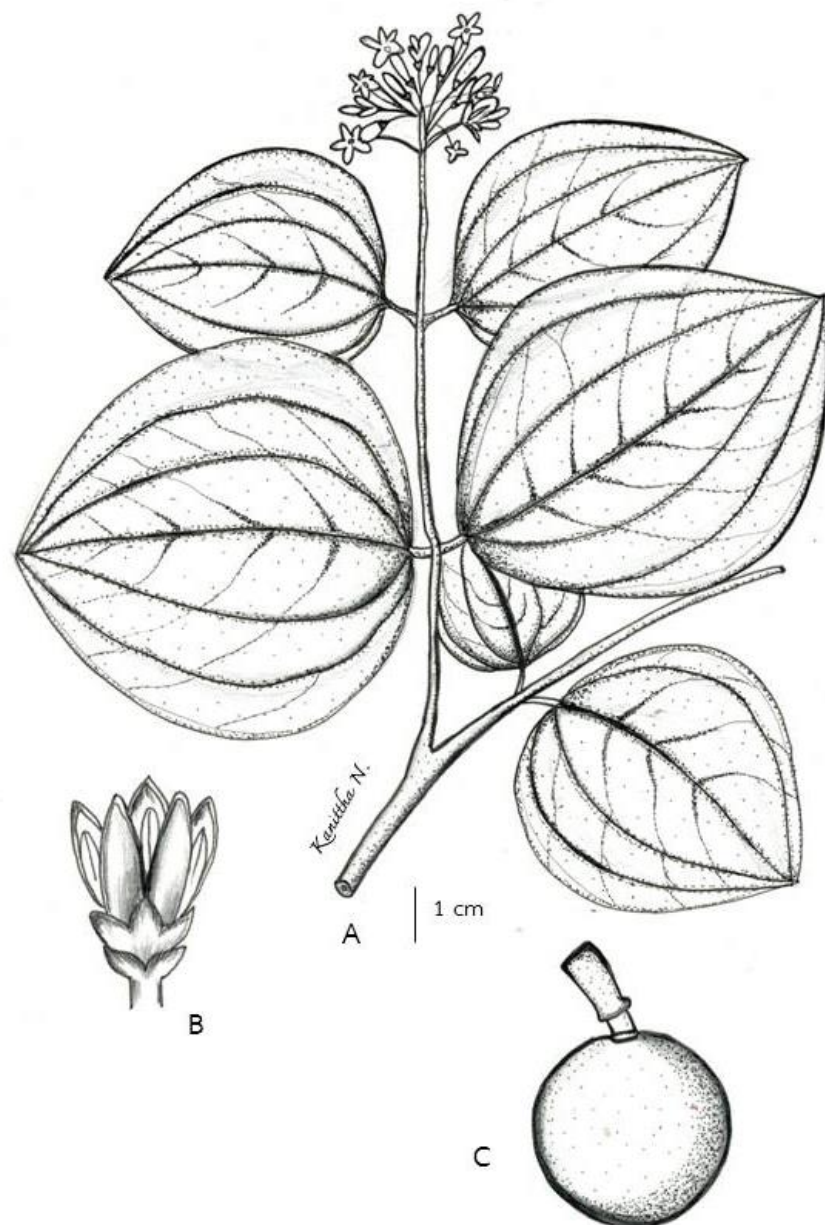
**Figure 25** The whole plant of *Strychnos nux-vomica* L. (A), flower (B) and berry (C)

*Strychnos nux-blanda* A. W. Hill

Leaves simple, opposite broadly ovate, 7 to 16 cm long. 9 to 22 cm wide, base broadly cuneate to cordate, apex gradually acute-acuminate. Laves are three to five veined from the base. Leaves of *S. nux-blanda* were shown in Figure 26. The whole plant of *S. nux-blanda* was demonstrated in Figure 27.



**Figure 26** Leaves of *Strychnos nux-blanda* A. W. Hill

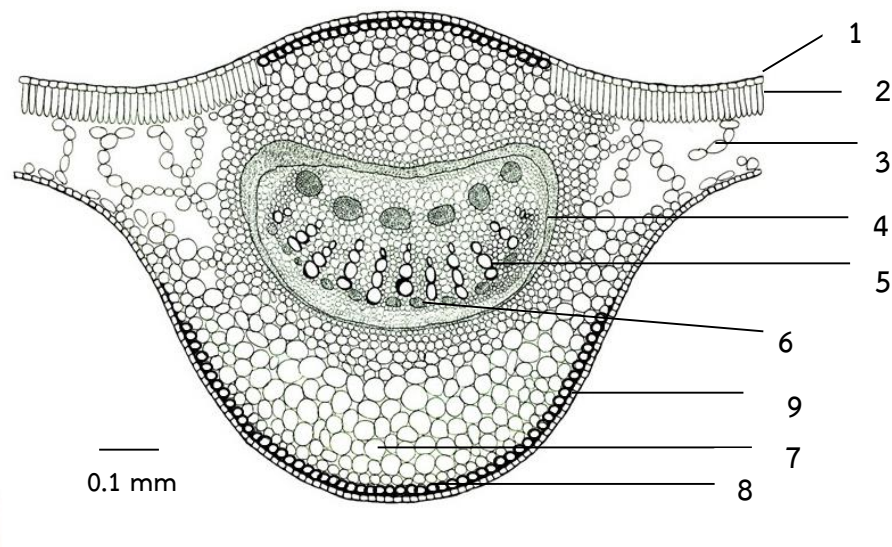


**Figure 27** The whole plant of *Strychnos nux-blanda* A. W. Hill (A)  
flower (B) and berry (C)

### Microscopic evaluation

Microscopic character of four *Strychnos* species were examined in both upper and lower epidermis, and transverse section of midrib. The outline drawings of midrib transvers sectional view of each species the feature characteristics were found and described as below

a) *S. thorelii*; the upper and lower surface is covered by single layer of epidermis. The upper epidermis showed the presence of well-developed cuticle without any trichomes. The midrib was composed of one collenchyma layer cells underneath the epidermis of both upper and lower epidermal. The mesophyll showed the presence of distinct one palisade layer and spongy parenchyma. Vascular bundles are conjoint, bi-collateral and exarch. All these vascular bundle are surrounded by sclerenchyma tissue as illustrated in Figure 28.

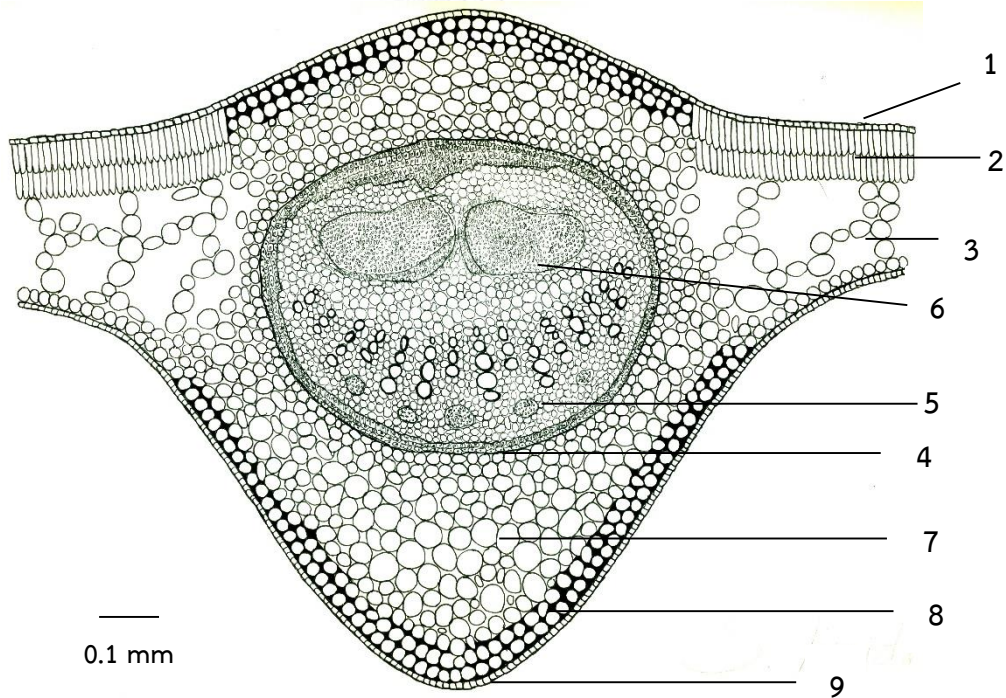


**Figure 28** The transverse section of *S. thorelii* leaf through midrib

1. Upper epidermis, 2. Palisade cell, 3. Spongy cell, 4. Sclerenchyma (fiber), 5. Xylem,
6. Phloem, 7. Parenchyma, 8. Collenchyma, 9. Lower epidermis



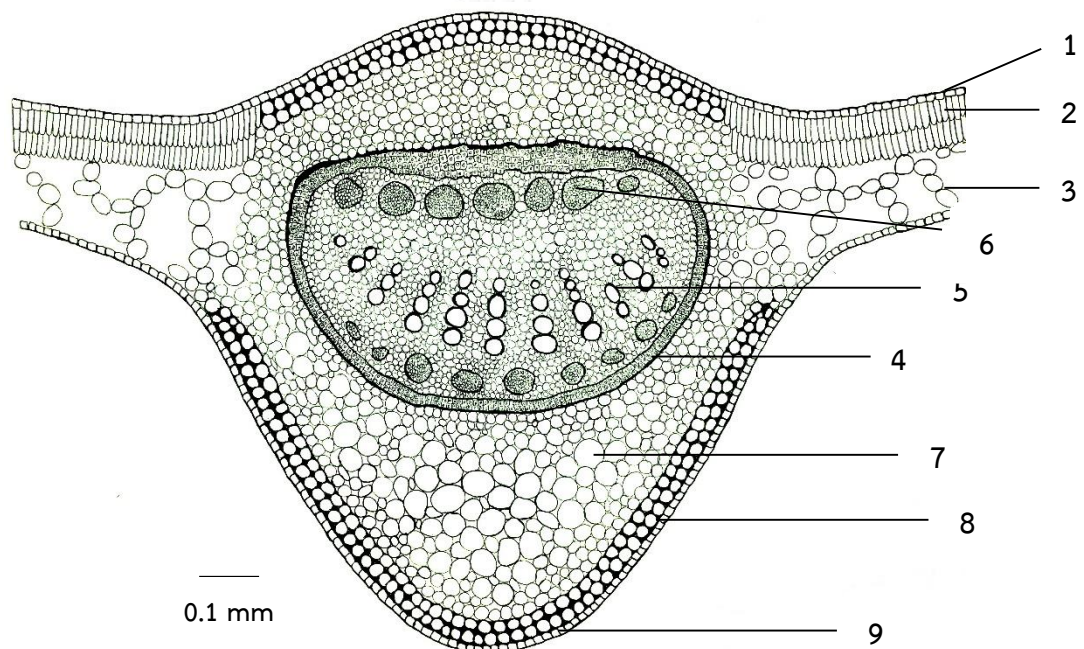
b) *S. lucida*; the upper and lower surface is covered by single layer of epidermis. The upper epidermis showed the presence of well-developed cuticle without any trichomes. The midrib was composed of 2-3 collenchyma layer cells underneath the epidermis of both upper and lower epidermal. The mesophyll was composed of two palisade layers and various spongy cells. Vascular bundles are conjoint, bi-collateral and exarch. The vascular bundles were towards the dorsal side two big vascular are also present. All these vascular bundle are surrounded by sclerenchyma tissue as shown in Figure 29.



**Figure 29** The transverse section of *S. lucida* leaf through midrib

1. Upper epidermis, 2. Palisade cell, 3. Spongy cell, 4. Sclerenchyma (fiber), 5. Xylem,  
6. Phloem, 7. Parenchyma, 8. Collenchyma, 9. Lower epidermis

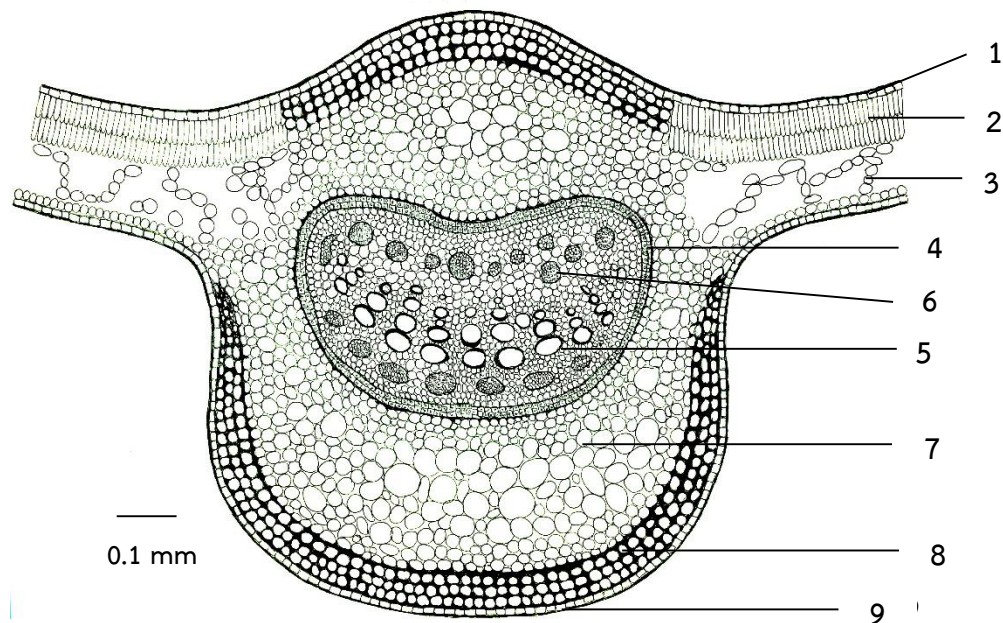
c) *S. nux-vomica*; the upper and lower surface is covered by single layer of epidermis. The upper epidermis showed the presence of well-developed cuticle without any trichomes. The midrib was composed of 2-3 collenchyma layer cells underneath the epidermis of both upper and lower epidermal. The mesophyll showed the presence of distinct two palisade layer and spongy parenchyma. Vascular bundles are conjoint, bi-collateral and exarch. All these vascular bundle are surrounded by sclerenchyma tissue as shown in Figure 30.



**Figure 30** The transverse section of *S. nux-vomica* leaf through midrib

1. Upper epidermis, 2. Palisade cell, 3. Spongy cell, 4. Sclerenchyma (fiber), 5. Xylem,  
6. Phloem, 7. Parenchyma, 8. Collenchyma, 9. Lower epidermis

d) *S. nux-blanda*; the upper and lower surface is covered by single layer of epidermis. The upper epidermis showed the presence of thick cuticle, without any trichomes. The midrib was composed of 3-4 collenchyma layer cells underneath the epidermis of both upper and lower epidermal. The mesophyll showed the presence of distinct two palisade layer and spongy parenchyma. Vascular bundles are conjoint, bi-collateral and exarch. All these vascular bundle are surrounded by sclerenchyma tissue as shown in Figure 31.

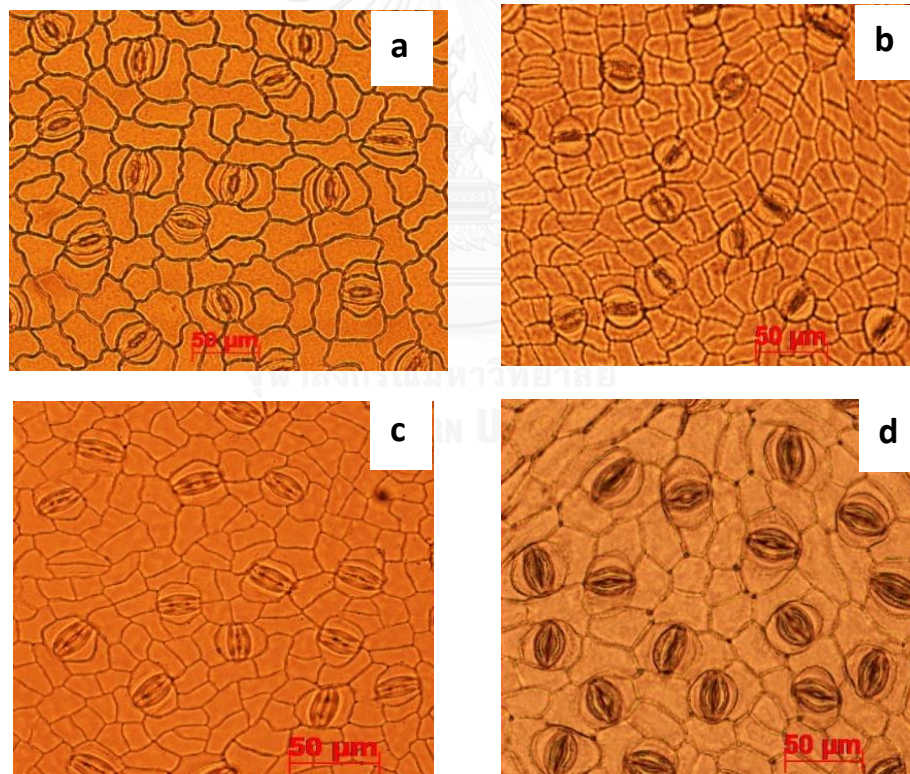


**Figure 31** The transverse section of *S. nux-blanda* leaf through midrib  
 1. Upper epidermis, 2. Palisade cell, 3. Spongy cell, 4. Sclerenchyma (fiber), 5. Xylem,  
 6. Phloem, 7. Parenchyma, 8. Collenchyma, 9. Lower epidermis

According to the following characteristics of midrib transverse section of each species, the xylem, phloem, parenchyma and sclerenchyma are arranged together to form a vascular bundle. There are different type of vascular bundle depending on arrangement of conducting tissues. Layer of palisade and collenchyma cell was different.

### The stomatal classification

The stoma type of four *Strychnos* species were classified as paracytic type, consisting of two subsidiary cells with the parallel to long axis of the stoma. The lower epidermis characteristics and of stomata among four *Strychnos* species were demonstrated in Figure 32.



**Figure 32** Paracytic type stoma of four *Strychnos* species

(a) *S. thorelii*, (b) *S. lucida*, (c) *S. nux-vomica* and (d) *S. nux-blanda*

### Leaf constant numbers

The leaf constant numbers consisting of stomatal number, stomatal index, palisade ratio, veinlet termination number, epidermal cell number and epidermal cell area by microscopic analyses were as mean  $\pm$ SD of each sample.

#### Stomatal number and stomatal index

The results of stomatal number and stomatal index of *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* from three different locations were illustrated in Table 9. The stomatal index among four species were different from each other with the highest value of *S. nux-blanda* ( $15.98 \pm 1.0$ ) and the lowest value of *S. lucida* ( $7.11 \pm 0.51$ ).

**Table 9** The stomatal number and stomatal index from four *Strychnos* species

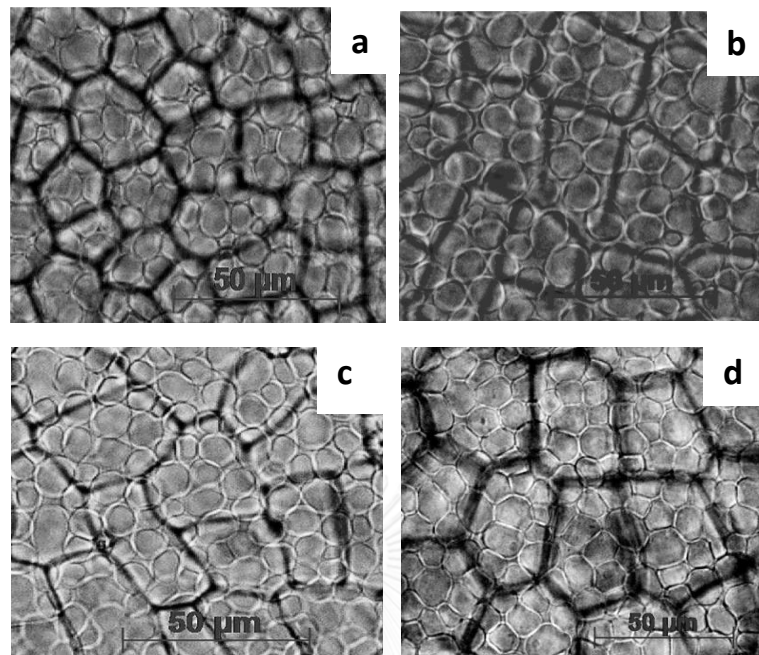
<i>Strychnos</i> species	Sample no.	Stomatal number (sq.mm)	Stomatal index
<i>S. thorelii</i>	1	154.27 $\pm$ 9.95	11.72 $\pm$ 0.66
	2	164.93 $\pm$ 11.51	11.51 $\pm$ 0.85
	3	166.67 $\pm$ 13.13	11.98 $\pm$ 0.99
	Avg.	161.96 $\pm$ 11.53	11.74 $\pm$ 0.83
<i>S. lucida</i>	1	167.47 $\pm$ 15.16	6.77 $\pm$ 0.73
	2	177.87 $\pm$ 14.16	7.52 $\pm$ 0.64
	3	170.40 $\pm$ 14.16	7.35 $\pm$ 0.50
	Avg.	171.91 $\pm$ 13.31	7.11 $\pm$ 0.51
<i>S. nux-vomica</i>	1	214.93 $\pm$ 14.13	8.71 $\pm$ 0.51
	2	202.93 $\pm$ 11.16	8.74 $\pm$ 0.48
	3	196.40 $\pm$ 11.95	8.56 $\pm$ 0.50
	Avg.	204.75 $\pm$ 12.41	8.67 $\pm$ 0.50
<i>S. nux-blanda</i>	1	239.33 $\pm$ 13.62	15.24 $\pm$ 0.68
	2	257.33 $\pm$ 18.03	16.44 $\pm$ 1.14
	3	254.93 $\pm$ 20.37	16.26 $\pm$ 1.18
	Avg.	250.53 $\pm$ 17.34	15.98 $\pm$ 1.0

### Palisade ratio

The palisade ratio of four *strychnos* species from three different locations were shown in Table 10. The highest value of palisade ratio was found in *S. nux-blanda* ( $12.29\pm 0.82$ ) and the lowest value was found in *S. thorelii* ( $4.42\pm 0.49$ ). The palisade cells of four *Strychnos* species was shown in Figure 33.

**Table 10** The palisade cells of four *Strychnos* species

<i>Strychnos</i> species	Sample no.	Palisade ratio
<i>S. thorelii</i>	1	$4.41\pm 0.48$
	2	$4.44\pm 0.48$
	3	$4.42\pm 0.58$
	Avg.	$4.42\pm 0.49$
<i>S. lucida</i>	1	$6.50\pm 0.73$
	2	$6.79\pm 0.78$
	3	$6.78\pm 0.69$
	Avg.	$6.69\pm 0.73$
<i>S. nux-vomica</i>	1	$7.98\pm 0.75$
	2	$9.36\pm 0.72$
	3	$9.45\pm 0.82$
	Avg.	$8.93\pm 0.76$
<i>S. nux-blanda</i>	1	$12.08\pm 0.81$
	2	$12.53\pm 0.81$
	3	$12.27\pm 0.85$
	Avg.	$12.29\pm 0.82$



**Figure 33** Palisade cell of four *Strychnos* species (a) *S. thorelii* (b) *S. lucida*  
(c) *S. nux-vomica* and (d) *S. nux-blanda*

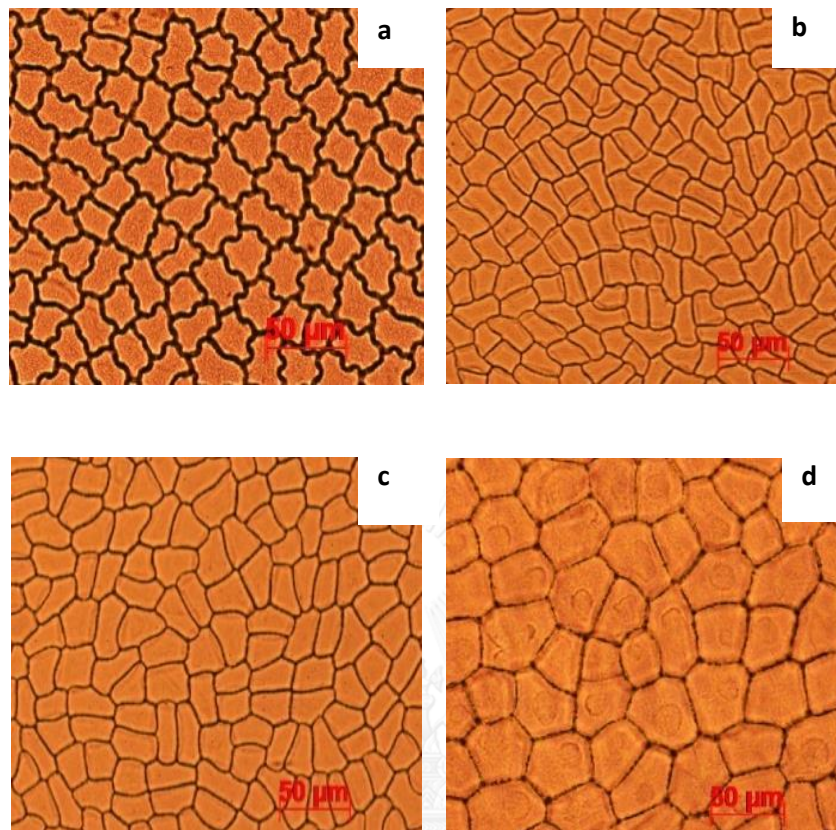
#### Epidermal cell number and epidermal cell area

Epidermal cell area was examined on upper epidermis among four *Strychnos* species. The highest epidermal cell area per square micrometer was found in *S. nux-blanda* ( $1102.71 \pm 24.74$ ). The lowest epidermal cell area was found in *S. lucida* ( $422.47 \pm 8.04$ ). The epidermal cell number and epidermal cell area of four *Strychnos* species from three locations was shown in Table 11 and the epidermal cells characteristics was shown in Figure 34.

**Table 11** The epidermal cell number and epidermal cell area from four *Strychnos* species

<i>Strychnos</i> species	Sample no.	Epidermal cell number (sq.mm)	Epidermal cell area (sq.µm)
<i>S. thorelii</i>	1	1628.40±37.89	614.31±13.85
	2	1648.93±38.25	606.77±14.00
	3	1658.67±46.26	598.83±12.89
	Avg.	1645.33±40.80	606.64±13.58
<i>S. lucida</i>	1	2386.35±47.57	419.32±8.32
	2	2349.75±45.05	425.75±8.10
	3	2368.53±43.23	422.34±7.69
	Avg.	2368.22±43.28	422.47±8.04
<i>S. nux-vomica</i>	1	1411.93±30.79	709.16±15.30
	2	1398.00±34.53	724.61±17.79
	3	1310.13±31.87	710.23±18.04
	Avg.	1374.35±32.40	711.05±17.04
<i>S. nux-blanda</i>	1	884.67±20.75	1130.95±25.82
	2	940.53±18.84	1063.64±21.57
	3	898.53±21.37	1113.56±26.82
	Avg.	907.91±20.37	1102.71±24.74





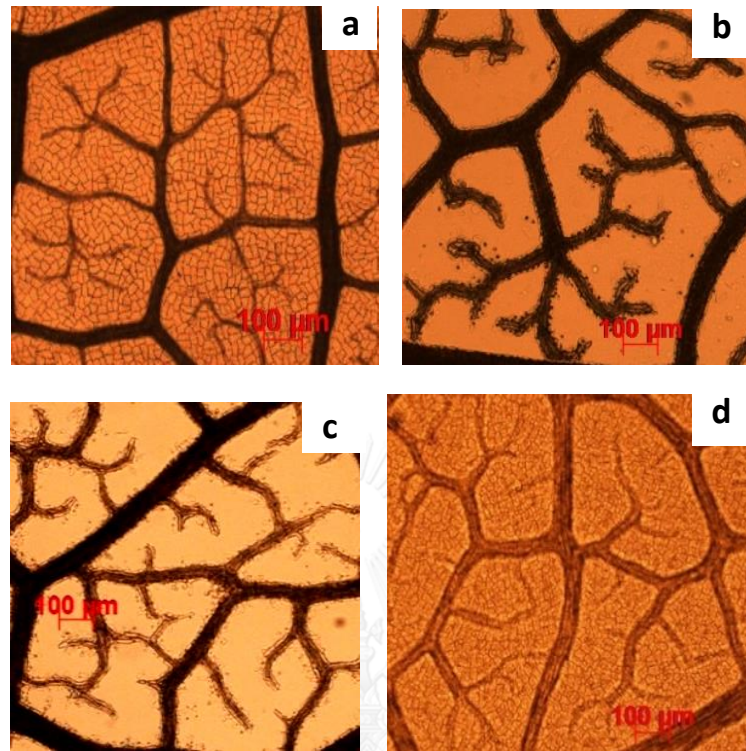
**Figure 34** Upper epidermis cell of four *Strychnos* species  
 (a) *S. thorelii*, (b) *S. lucida*, (c) *S. nux-vomica* and (d) *S. nux-blanda*

### Veinlet termination number

Veinlet termination numbers of four *Strychnos* species were shown in Table 12. The highest veinlet termination number was found in *S. nux-blanda* ( $17.93 \pm 1.75$ ) and the lowest epidermal cell area was found in *S. thorelii* ( $15.47 \pm 1.13$ ), the veinlet termination characters were in Figure 35.

**Table 12** The veinlet termination number from four *Strychnos* species

<i>Strychnos</i> species	Sample no.	Veinlet termination number (sq.mm)
<i>S. thorelii</i>	1	16.18±1.14
	2	14.57±1.13
	3	15.65±1.13
	Avg.	15.47 ±1.13
<i>S. lucida</i>	1	16.42±1.51
	2	16.66±1.63
	3	16.63±1.96
	Avg.	16.57±1.80
<i>S. nux-vomica</i>	1	19.22±1.84
	2	17.06±1.65
	3	17.78±1.95
	Avg.	17.93±1.75
<i>S. nux-blanda</i>	1	16.35±1.66
	2	16.92±1.29
	3	15.49±1.21
	Avg.	16.25±1.39



**Figure 35** Venlet termination of four *Strychnos* species

(a) *S. thorelii* (b) *S. lucida* (c) *S. nux-vomica* and (d) *S. nux-blanda*

The summary of leaf constant numbers consisting of stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and venlet termination number of four *Strychnos* species were shown in Table 13 and the raw data of each parameters were shown in appendix A.

**Table 13** Summary of the leaf constant numbers consisting of stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number from four *Strychnos* species

<i>Strychnos</i> species	Sample no.	Stomatal number (sq.mm)	Stomatal index	Palisade ratio	Epidermal cell number (sq.mm)	Epidermal cell area (sq.µm)	Veinlet termination number (sq.mm)
<i>S. thorelii</i>	1	154.27±9.95	11.72±0.66	4.41±0.48	1628.40±37.89	614.31±13.85	16.18±1.14
	2	164.93±11.51	11.51±0.85	4.44±0.48	1648.93±38.25	606.77±14.00	14.57±1.13
	3	166.67±13.13	11.98±0.99	4.42±0.58	1658.67±46.26	598.83±12.89	15.65±1.13
	avg.	161.96±11.53	11.74±0.83	4.42±0.49	1645.33±49.54	606.64±13.58	15.47±1.13
<i>S. lucida</i>	1	167.47±15.16	6.77±0.73	6.50±0.73	2386.35±47.57	419.32±8.32	16.42±1.51
	2	177.87±14.16	7.52±0.64	6.79±0.78	2349.75±45.05	425.75±8.10	16.66±1.63
	3	170.40±14.16	7.35±0.50	6.78±0.69	2368.53±43.23	422.34±7.69	16.63±1.96
	avg.	171.91±13.31	7.11±0.51	6.69±0.73	2368.22±43.28	422.47±8.04	16.57±1.80
<i>S. nux-vomica</i>	1	214.93±14.13	8.71±0.51	7.98±0.75	1411.93±30.79	709.16±15.30	19.22±1.84
	2	202.93±11.16	8.74±0.48	9.36±0.72	1398.00±34.53	724.61±17.79	17.06±1.65
	3	196.40±11.95	8.56±0.50	9.45±0.82	1310.13±31.87	710.23±18.04	17.78±1.95
	avg.	204.75±12.41	8.67±0.50	8.93±0.76	1374.35±32.40	711.05±17.04	17.93±1.75
<i>S. nux-blanda</i>	1	239.33±13.62	15.24±0.68	12.08±0.81	884.67±20.75	1130.95±25.82	16.35±1.66
	2	257.33±18.03	16.44±1.14	12.53±0.81	940.53±18.84	1063.64±21.57	16.92±1.29
	3	254.93±20.37	16.26±1.18	12.27±0.85	898.53±21.37	1113.56±26.82	15.49±1.21
	avg.	250.53±17.34	15.98±1.0	12.29±0.82	907.91±20.37	1102.71±24.74	16.25±1.39

### Molecular evaluation

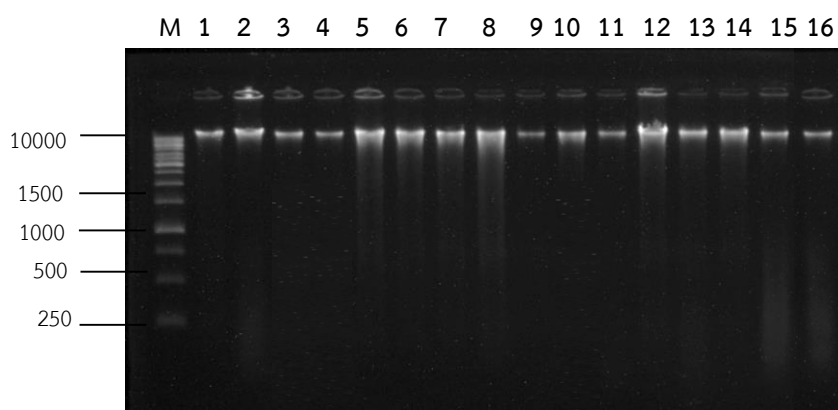
Young fresh leaves of four *Strychnos* species were collected from 16 different locations in Thailand as shown in Table 14.

**Table 14** List of four *Strychnos* species collected from 16 different locations in Thailand

<i>Strychnos</i> species	Sample no	Location	Collecting date	Voucher ID
<i>S. thorelii</i>	1	Chachoengsao	Aug, 2014	St1
	2	Chachoengsao	Aug, 2014	St2
	3	Chonburi	Oct, 2014	St3
	4	Chonburi	Oct, 2014	St4
<i>S. lucida</i>	1	Bangkok	Sep,2014	Sl1
	2	Pathum Thani	Sep,2014	Sl2
	3	Nonthaburi	Feb, 2015	Sl3
<i>S. nux-vomica</i>	1	Pathum Thani	Sep, 2014	Sv1
	2	Khon Kaen	Nov, 2014	Sv2
	3	Songkhla	Nov, 2014	Sv3
	4	Chachoengsao	Dec, 2014	Sv4
	5	Chanthaburi	Feb, 2015	Sv5
<i>S. nux- blanda</i>	1	Chiang Mai	Nov, 2014	Sb1
	2	Chiang Mai	Nov, 2014	Sb2
	3	Chiang Mai	Nov, 2014	Sb3
	4	Chiang Mai	Nov, 2014	Sb4

### The results of DNA extraction

The total genomic DNA was individually isolated from young leaves using modified CTAB method as described in chapter 3. The genomic DNA was examined on 1.5% agarose gel electrophoresis after staining with ethidium bromide as shown in Figure 36. The extracted total DNA was then stored at -20 °C for further use as DNA templates in PCR amplification.



**Figure 36** 1.5% agarose gel electrophoresis of genomic DNA *Strychnos* species

Lane M: 1 Kb DNA ladder

Lane 1-4: *S. thorelii*

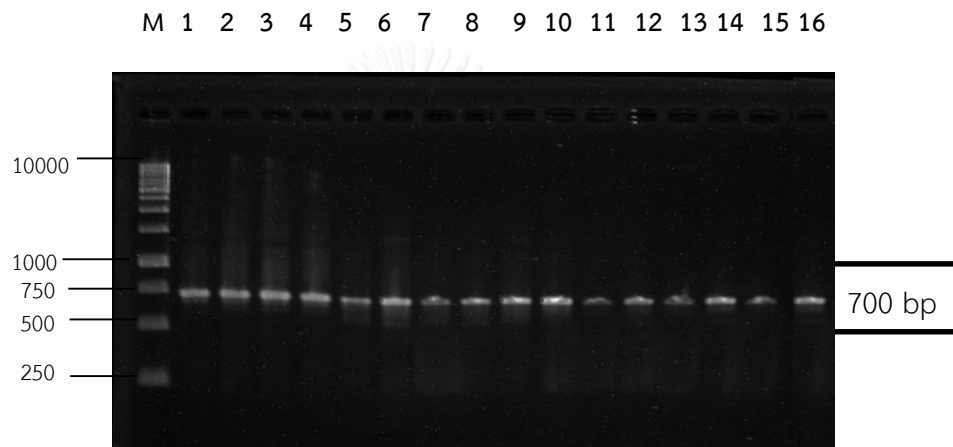
Lane 5-7: *S. lucida*

Lane 8-12: *S. nux-vomica*

Lane 13-16: *S. nux-blanda*

### PCR amplifications of ITS region, *rbcl* and *matK* gene

For amplification of ITS region, a pair of universal PCR primers (ITS5 and ITS4) designed from highly conserved regions flanking the Internal transcribe spacer (ITS) region were used for PCR amplification. The PCR products were dispersed in 1.5% agarose gel electrophoresis. When compared to 1 kb DNA ladder the PCR products were approximately 700 base pairs (bp) in size as shown in Figure 37.



**Figure 37** PCR products of ITS on 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV transilluminator

Lane M: 1 Kb DNA ladder

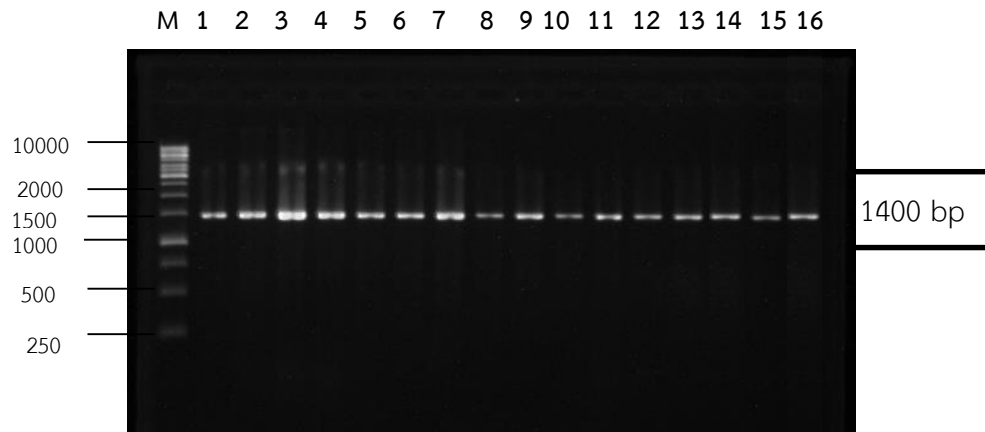
Lane 1-4: *S. thorelii*

Lane 5-7: *S. lucida*

Lane 8-12: *S. nux-vomica*

Lane 13-16: *S. nux-blanda*

For amplification of *rbcl* gene, a pair of specific *rbcl* PCR primers was used for *rbcl* gene for PCR amplification. The PCR products were dispersed in 1.5% agarose gel electrophoresis. When compared to 1 kb DNA ladder, the PCR products were approximately 1400 bp in size as shown in Figure 38.



**Figure 38** PCR products of *rbcl* gene on 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV transilluminator

Lane M: 1 Kb DNA ladder

Lane 1-4: *S. thorelii*

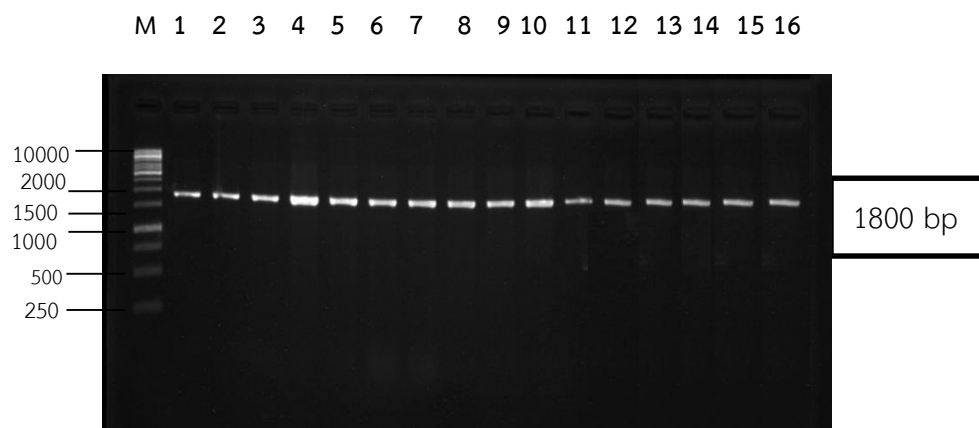
Lane 5-7: *S. lucida*

Lane 8-12: *S. nux-vomica*

Lane 13-16: *S. nux-blanda*



For amplification of *matK* gene, a pair of specific *matK* PCR primer was used for amplification. The PCR products were dispersed in 1.5% agarose gel electrophoresis. When compared to 1 kb DNA ladder, the PCR products gene were approximately 1800 bp in size shown in Figure 39.



**Figure 39** PCR products of *matK* gene on 1.5% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV transilluminator

Lane M: 1 Kb DNA ladder

Lane 1-4: *S. thorelii*

Lane 5-7: *S. lucida*

Lane 8-12: *S. nux-vomica*

Lane 13-16: *S. nux-blanda*

### DNA sequencing

The raw data of DNA sequences of ITS region, *rbcl* gene and *matK* gene from *S. thorelii*, *S. lucida*, *S. nux-vomica* and *S. nux-blanda* were included in Appendix B.

The length of the ITS sequence among four *Strychnos* species were 730-738 bp. The similarity of the ITS region nucleotide sequence among the intra-species range from 98 % to 99% while the inter-species range from 87% to 99%.

The length of the *rbcl* sequence among four *Strychnos* species, were from 1440 to 1463 bp. The similarity of the *rbcl* nucleotide sequence among the intra-species range from 95 % to 99% while the inter-species range from 94% to 97%.

The length of the *matK* sequence among four *Strychnos* species 1808 to 1815 bp in length. The similarity of the *matK* nucleotide sequence among the intraspecies range from 96 % to 99% while the interspecies range from 90% to 95%.

### PCR-RFLP method for species differentiation using *matK* gene

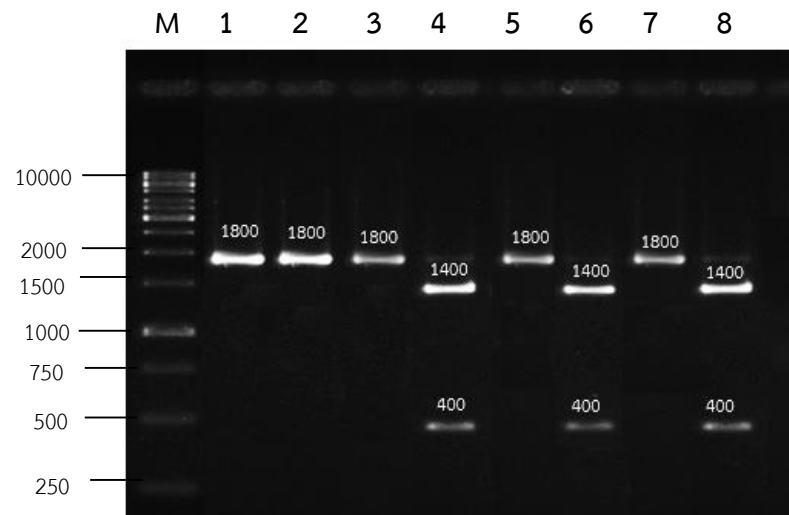
Based on the alignment of the *matK* gene of four *Strychnos* species, the polymorphic nucleotides were observed at different positions as shown in Figure 42. The PCR products were digested with two restriction enzymes, *DraI* and *XbaI*. The restriction fragments were separated on 1% agarose gel electrophoresis along with the 1 Kb ladder molecular marker. The PCR-RFLP pattern of the *matK* gene was shown in Figure 40-41. When the PCR product was digested with *XbaI*, the PCR-RFLP restriction pattern can be distinguished *S. thorelii* from the other three *Strychnos* species by observing the single uncut 1800 bp band while the other three *Strychnos* species shows the 1400 and 400 bp bands as shown in Figure 40 and Table 15.

To distinguish the three *Strychnos* species, the PCR product was digested with *DraI* restriction enzyme. The PCR-RFLP restriction pattern can be distinguished *S. nux-vomica*, *S. lucida* and *S. nux-blanda*.

In *S. nux-vomica*, there are two bands of 1620 and 180 fragments in size were observed after digestion with *DraI*. For *S. lucida*, there are three bands of 1150, 360 and 290 fragments in size while *S. nux-blanda* showed the three bands of 1210, 380 and 210 fragments in size after digestion with *DraI* restriction enzyme as shown in Figure 41 and Table 15.

**Table 15** The size of restriction fragment of four *Strychnos* species digest with *XbaI* and *DraI*

<i>Strychnos</i> Species	Restriction fragment (base pair)	
	<i>XbaI</i>	<i>DraI</i>
<i>S. thorelii</i>	1800	1210, 380, 210
<i>S. lucida</i>	1400, 400	1150, 360, 290
<i>S. nux-vomica</i>	1400, 400	1620, 180
<i>S. nux-blanda</i>	1400, 400	1210, 380, 210



**Figure 40** PCR-RFLP pattern of four *Strychnos* species digested with *Xba*I

Lane M: 1 Kb DNA ladder

Lane 1: undigested PCR product of *S. thorelii*

Lane 2: *Xba*I digested PCR product of *S. thorelii*

Lane 3: undigested PCR product of *S. nux-vomica*

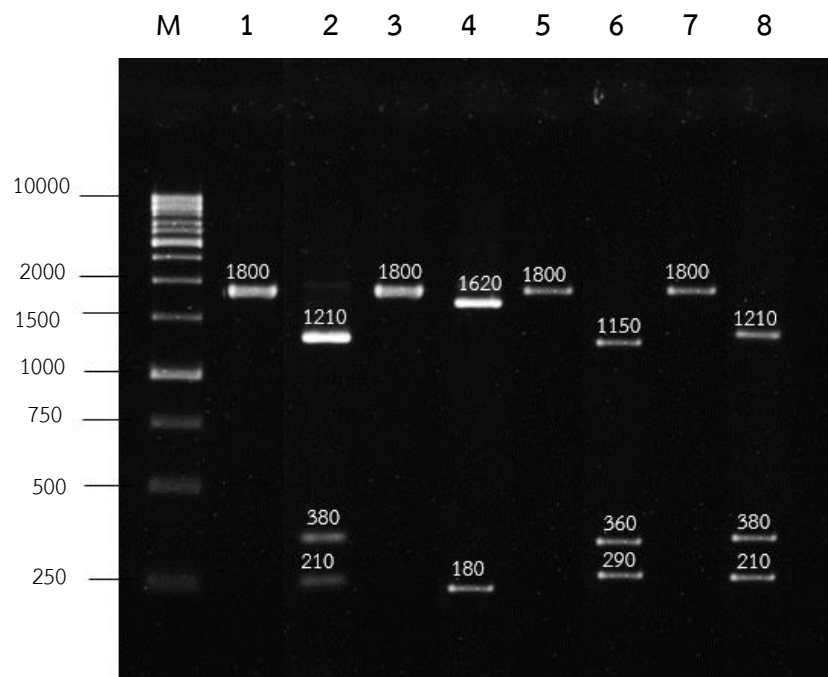
Lane 4: *Xba*I digested PCR product of *S. nux-vomica*

Lane 5: undigested PCR product of *S. lucida*

Lane 6: *Xba*I digested PCR product of *S. lucida*

Lane 7: undigested PCR product of *S. nux-blanda*

Lane 8: *Xba*I digested PCR product of *S. nux-blanda*



**Figure 41** PCR-RFLP pattern of four *Strychnos* species digested with *Dral*

Lane M: 1 Kb DNA ladder

Lane 1: undigested PCR product of *S. thorelii*

Lane 2: *Dral* digested PCR product of *S. thorelii*

Lane 3: undigested PCR product of *S. nux-vomica*

Lane 4: *Dral* digested PCR product of *S. nux-vomica*

Lane 5: undigested PCR product of *S. lucida*

Lane 6: *Dral* digested PCR product of *S. lucida*

Lane 7: undigested PCR product of *S. nux- blanda*

Lane 8: *Dral* digested PCR product of *S. nux- blanda*

<i>S. lucida</i>	ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTTCTATA	59
<i>S. nux-vomica</i>	ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGGGGCTTTCTATA	60
<i>S. thorelii</i>	ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTTCTATA	59
<i>S. nux-blanda</i>	ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTTCTATA *****	59
<i>S. lucida</i>	TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGCTTAAA	118
<i>S. nux-vomica</i>	TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGCTTAAA	119
<i>S. thorelii</i>	TCAACACTTCTCTTTCAAGAAGAGTATATTTTATGCACCTGCTCATGATCATAGCTTAAA	119
<i>S. nux-blanda</i>	TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGCTTAAA ***** * * * * *	118
<i>S. lucida</i>	AACGGATCTATATTTGTTGGACGGGAATTTCCAGATT-TTGTGGACAACCC-AGGTTAGT	176
<i>S. nux-vomica</i>	AACGGATCTATATTTGTTGGGAAGGAAAAGCCAGGCT-TTTTGGAAAATCC-AGGTAAT	177
<i>S. thorelii</i>	AACGGATCTATATTTGTTGGGAAGGAAAATCCAGGTT-ATGATGGAAAATCC-AGGTTAGT	177
<i>S. nux-blanda</i>	AACGGATCTATATTTGTTGAAAGGGAAAATCCCGGTT-TTGTGGAAAATCC-AGGTTAGT ***** * * * * *	176
<i>S. lucida</i>	TGATAATAAATCCAAAACGTTTTCTAAGATTGTGAAAATCCGTGTTAATTACTCGAATGT	236
<i>S. nux-vomica</i>	CGATAATAAGATCAAAACGTTATCTAAGGTGGTGAACACTAGTTTTTAATTACTCAAATGT	237
<i>S. thorelii</i>	AGATACTAATTGTAAAACGTTTTCTAGGACTGTGTAATTACTGTTTAATTACGCGAATGT	237
<i>S. nux-blanda</i>	TGATAATAAATCCAAAACGTTTTCTAAGATTGTGAAAATCCGTGTTAATTACTCGAATGT * * * * *	236
<i>S. lucida</i>	ATCAACAGTTAAATCATTTCTTTATGATACAA--GGTTTTCTAATGATTTCTAAACAAAA	294
<i>S. nux-vomica</i>	AAAAACATTTAAGTCAAGTTTACAAGATTTTTTGTATTTTCTAATGATTTCTAAACAAAA	297
<i>S. thorelii</i>	ATCAACAGTTAAATCATTTTTTTATGATACAA--GGTTTTCTAATGATTTCTAAGCAAAA	295
<i>S. nux-blanda</i>	ATCAACAGTTAAATCATTTTTTTATGATACAA--GGTTTTCTAATGATTTCTAAACAAAA ***** * * * * *	294
<i>S. lucida</i>	TCGATTTTTATTTTTGGGCCACAGCAAGAATTGGTATTACCAATGATATCAAAGGGATT	354
<i>S. nux-vomica</i>	TCGATTTTTATTTTTGGGGCACAGCAAGAATTGTATTCTCAAATGATATCAGAGGGATT	357
<i>S. thorelii</i>	TCGATTTTTATTTTTGGGGCACAGCAAGAATTGTATTCTCAAATGATATCAGAGGGATT	355
<i>S. nux-blanda</i>	TCGATTTTTATTTTTGGGCCACAGCAAGAATTGTATTCCCAAATGATATCCGAGGGATT ***** * * * * *	354
	▽ <i>Dra</i> I	▽ <i>Xba</i> I
<i>S. lucida</i>	TTCCCTTATTGTGGAAT-TCGTTTTCTAAACGGATTACTATCTTCT <b>TCTAGAGA</b> AGAAG	413
<i>S. nux-vomica</i>	TTCCCTTATTGTGGAATGTCGTTTTCTAGAAGGATTACTATCTTCT <b>TCTAGAGA</b> AGAAA	417
<i>S. thorelii</i>	TTCCCTTATTGTGGAAT-TCGTTTT <b>TTTAAAC</b> CGGATTACTATCTTCTTCTATAGAAGAAG	414
<i>S. nux-blanda</i>	TCCCTTATTGTGGAAT-TCGTTTT <b>TTTAAAC</b> CGGATTACTATCTTCT <b>TCTAGAGA</b> AGAAG * * * * *	413
<i>S. lucida</i>	AGGAAAGGGGTATTCAAATTACAGAATTCACATTTATGCTCAATTCATCAATATTTCT	473
<i>S. nux-vomica</i>	GGGAAAGGGGTATTCAAATTACAGAATTCACATTTATGCTCAATTCATCAATATTTCT	477
<i>S. thorelii</i>	AGGAAAGGGGTATTCAAATTACAGAATTCACATTTACGCTCAATTCATCAATATTTCT	474
<i>S. nux-blanda</i>	AGGAAAGGGGTATTCAAATTCAGAATTCACATTCAGCCCAATTCATCAATATTTCT *****	473
<i>S. lucida</i>	TTCTTAGAGGACAACCTTTTCACATCTCAATTTATGTGTTAGATATACTAATACCCCACCC	533
<i>S. nux-vomica</i>	TTCTTAGAGGACAACCTTTTCACATCTCAATTTATGTGTTAGATATACTAATACCCCACCC	537
<i>S. thorelii</i>	TTCTTAGAGGACAACCTTTTCACATCTCAATTTATGTGTTAGATATACTAATACCCCACCC	534
<i>S. nux-blanda</i>	TTCTTAGAGGACAACCTTTTCACATCTCAATTTATGTGTTAGATATACTAATACCCCACCC *****	533
<i>S. lucida</i>	GTACATCTGGAATCCTGGTTCAAACCCCTCGCTATTGGGTAAAAGATGCCTCTTCTTTG	593
<i>S. nux-vomica</i>	GTACATCTGGAATCCTGGTTCAAACCCCTCGCTATTGGGTAAAAGATGCCTCTTCTTTG	597
<i>S. thorelii</i>	GTCCATCTGGAATCCTGGTTCAAACCCCTCGCTATTGGGTAAAAGATGCCTCTTCTTTG	594
<i>S. nux-blanda</i>	GTCCATCTGGAATCCTGGTTCAAACCCCTCGCTAAATGGGTAAAAGATGCCTCTTCTTTG *****	593
<i>S. lucida</i>	CATTTATTACGATTCTTTCTCTACGAGTATTGTAATTATTGTAATTGGAATAATCTTATT	653
<i>S. nux-vomica</i>	CATTTATTACGATTCTTTCTCTACGAGTATTGTAATTATTGTAATTGGAATAATCTTATT	657
<i>S. thorelii</i>	CATTTATTACGATTCTTTCTCTACGAGTATTGTAATTATTGTAATTGGAATAATCTTATT	654
<i>S. nux-blanda</i>	CATTTATTACGATTCTTTCTCTACGAGTATTGTAATTATTGTAATTGGAATAATCTTATT *****	653

<i>S. lucida</i>	TCTACAAAGAAACCCAGTTTTCTTTTTTAACAAAAAGAAATAAAAGATTATTCTTCTTC	713
<i>S. nux-vomica</i>	TCTACAAAGAAACCCAGTTTTCTTTTTTAACAAAAAGAAATAAAAGATTATTCTTCTTC	717
<i>S. thorelii</i>	GCTACAAAGAAACCCAGTTTTCTTTTTTACCAAAAAGAAATAAAAGATTATTCTTCTTC	714
<i>S. nux-blanda</i>	TCTACAAAGAAACCCAGTTTTCTTTTTTAACAAAAAGAAATAAAAGATTATTCTTCTTC	713
	*****	
<i>S. lucida</i>	TTAAATAATTCTTAAGTATGTGAATACGAATCCATTTTCGCTTTCTACATAACCAATCT	773
<i>S. nux-vomica</i>	TTAAATAATTCTTAATAAGGTCAATACGAATCCATTTTCGCTTTCTACATAACCAATCT	777
<i>S. thorelii</i>	TTATATAATTCTTATGTATGTGAATACGAATCCATTTTCCTCTTTCTACATAACCAATCT	774
<i>S. nux-blanda</i>	TTAAAAATCCTTAAGTATGTGAATACGAATCCATTTTCGCTTTCTACATAACCAATCT	773
	*** * ** *	
<i>S. lucida</i>	TCTCATTACGATCAACATCCTTTGAGGCCCTTCTGAACGAATCCATTTCTATGGAAAA	833
<i>S. nux-vomica</i>	TCTCATTACGATCAACATCCTTTGAGGCCCTTCTGAACGAATCCATTTCTATGGAAAA	837
<i>S. thorelii</i>	TCTCATTACGATCAACATCCTTTGAGGTCCTTCTGAACGAATCTATTTCTATGGAAAA	834
<i>S. nux-blanda</i>	TCTCATTACGATCAACATCCTTTGAGGTCCTTCTGAACGAATCCATTTCTATGGAAAA	833
	*****	
<i>S. lucida</i>	ATAGAACGCTTTGTAGAAGTCTTTGCTAAGGATTATCAGGCCAAACTATGGTTGTTCAAA	893
<i>S. nux-vomica</i>	ATAGAACGCTTTGTAGAAGTCTTTGCTAAGGATTATCAGGCCAAACTATGGTTGTTCAAA	897
<i>S. thorelii</i>	ATAGAACGCTTTGTAGAAGTCTTTGCTAAGGATTATCAAGCCAAACTACGGTTGTTCAAA	894
<i>S. nux-blanda</i>	ATAGAACGCTTTGTAGAAGTCTTTGCTAAGGATTATCAGGCCAAACTATGGTTGTTCAAA	893
	*****	
<i>S. lucida</i>	GATCCTTTCATACATTATGTTAGGTATCAAGGAAAATTCATTCTGGTTTCAAAGGGGACG	953
<i>S. nux-vomica</i>	GATCCTTTCATACATTATGTTAGGTATCAAGGAAAATTCATTCTGGTTTCAAAGGGGACG	957
<i>S. thorelii</i>	GATCCTTTCATACATTATGTTAGGTATCAAGGAAAATTCATTCTGGTTTCAAAGGGGACG	954
<i>S. nux-blanda</i>	GATCCTTTCATACATTATGTTAGGTATCAAGGAAAATTCATTCTGGTTTCAAAGGGGACG	953
	*****	
<i>S. lucida</i>	GCTCCTTTGATGAATAAATGGAATCTTACCTTGGCAATTTTTGGCAATGTCATTTTGAC	1013
<i>S. nux-vomica</i>	GCTCCTTTGATGAATAAATGGAATCTTACCTTGTCAATTTTTGGCAATGTCATTTTGAC	1017
<i>S. thorelii</i>	GCTCCTTTGATGAATAAATGGAATCTTACCTTGTCAATTTTTGGCAATGGCATTTTGAC	1014
<i>S. nux-blanda</i>	GCTCCTTTGATGAATAAATGGAATCTTACCTTGGCAATTTTTGGCAATGTCATTTTGAC	1013
	*****	
<i>S. lucida</i>	CTGGGGTTTCACTCCGGAAGGGTCTATATAAAGGAATTATACCAATCATTCCTTGGAC	1073
<i>S. nux-vomica</i>	CTGGGGTTTCACTCCGGAAGGGTCTATATAAAGGAATTATACCAATCATTCCTTGGAC	1077
<i>S. thorelii</i>	CTGGGGTTTCACTCCGGAAGGGTCTATATAAAGGAATTATACCAATCATTCCTTGGAC	1074
<i>S. nux-blanda</i>	CTGGGGTTTCACTCCGGAAGGGTCTATATAAAGGAATTATACCAATCATTCCTTGGAC	1073
	*****	
<i>S. lucida</i>	TTTATGGGGCTATCTTTCAGAAGGGGTCGACTAAACCCTTTCAATGGGTACGGGAGTCC	1133
<i>S. nux-vomica</i>	TTTATGGGGCTATCTTTCAGAAGTGGCGCTTAAACCCTTTCAATGGGTACGGGAGTCC	1137
<i>S. thorelii</i>	TTTATGGGGCTATCTTTCAGAAGTGGCGACTAAACCCTTTCAATGGGTACGGGAGTCC	1134
<i>S. nux-blanda</i>	TTTATGGGGCTATCTTTCAGAAGGGGGCGACTAAACCCTTTCAATGGGTACGGGAGTCC	1133
	*****	
	▽ <i>DraI</i>	
<i>S. lucida</i>	AAATGAAAGAATTTAAACATTTCC-TAAACCAATAATGCCATTAGGAAAAAATGGG	1192
<i>S. nux-vomica</i>	AAATGAAAGAATTAATTCATTTTC-TAATCCAATAAGGGCTATTATAGGAAAAATGGG	1196
<i>S. thorelii</i>	AAATGAAAGAATTAACATTTCCCTAATCCAATAATGGCCTTTTAGGAAAAAATGGG	1194
<i>S. nux-blanda</i>	AAATGAAAGAATTAACATTTTCCCAACCAAAAAGGCCTATTAGGAGAAAAATGGG	1193
	***** * **** * * * * *	
<i>S. lucida</i>	AAACCCCTTGGTCCAAAAATTATTTCCCTCCTGGGGGTGGGACCATTGGTGGTTAAACC	1252
<i>S. nux-vomica</i>	AAACCCCTTGGTCCAAAAATTATTTCCCTCCTGGGTGTGGGAAACATATGGGGTTATAAGC	1256
<i>S. thorelii</i>	AAACCCCTTGGTCCAAAAATTATCCCCCTTGGGGGTGGGAACATATGGGGTTAAAGC	1254
<i>S. nux-blanda</i>	AAACCCCTTGGTCCAAAAATTATTTCCCTCCTGGGGGTGGGAACATATGGGGTTAAAGC	1253
	***** * ** * * * * *	
<i>S. lucida</i>	CAAAAGTTTGTGAACCCACCTAAGGGGCATCCCATTTAGTTAAAGCCGGTTTGGACTGAT	1312
<i>S. nux-vomica</i>	GAAAAGTTTGGTAACCCATAGGGGGGCATCCCATTTAGTTAAAGCCGGTTTGGACTGAT	1316
<i>S. thorelii</i>	GAAATTTTTGGAAACCCCTTAGGGGGGCATCCCATTTAGTTAAAGCCGGTTTGGACTGAT	1314
<i>S. nux-blanda</i>	GAAATTTTTGGTAACCCATAGGGGGGCATCCCATTTAGTTAAAGCCGGTTTGGACTGAT	1313
	*** ** * * * *	

<i>S. lucida</i>	TTATCAGATTCGGATATTATTGACCGATTTGGGCGTATATGCAGAAATCTTTCTCATTAT	1372
<i>S. nux-vomica</i>	TTATCAGATTCGGATATTATTGACCGATTTGGGCGTATATGCAGAAATCTTTCTCATTAT	1376
<i>S. thorelii</i>	TTATCAGATTCGGATATTATTGACCGGGTTGGGCGTATATGCAGAAATCTTTCTCATTAT	1374
<i>S. nux-blanda</i>	TTATCAGATTCGGATATTATTGACCGATTTGGGCGTATATGCAGAAATCTTTCTCATTAT	1373
*****		
<i>S. lucida</i>	CATAGCGGATCTTCCAAAAAAGAGTTTGTATCGAATAAAGTATATACTTCGGCTTTCT	1432
<i>S. nux-vomica</i>	CATAGTGGATCTTCCAAAAAAGAGTTTGTATCGAATAAAGTATATACTTCGGCTTTCT	1436
<i>S. thorelii</i>	CATAGCGGATCTTCCAAAAAAGTGTAGTTTGTATCGAATAAAGTATATACTTCGGTTTTCT	1434
<i>S. nux-blanda</i>	CATAGCGGATCTTCCAAAAAAGAGTTTGTATCGAATAAAGTATATACTTCGGCTTTCT	1433
*****		
<i>S. lucida</i>	TGTGCTAAAATTTTAGCTCGGAAACACAAAAGTACTGTACGTGCTTTTTTGAAAAGATTA	1492
<i>S. nux-vomica</i>	TGTGCTAAAACCTTTAGCTCGGAAACACAAAAGTACTGTACGTGCTTTTTTGAAAAGATTA	1496
<i>S. thorelii</i>	TGTGCTAAAACCTTTAGCTCGGAAACACAAAAGTACTGTACGTGCTTTTTTGAAAAGATTA	1494
<i>S. nux-blanda</i>	TGTGCTAAAACCTTTAGCTCGGAAACACAAAAGTACTGTACGTGCTTTTTTGAAAAGATTA	1493
*****		
▽ <i>DraI</i>		
<i>S. lucida</i>	GGGTCGGAATTTTGGAA <b>TTTAA</b> ACTCATGTGCGGAAGAAGTAGCCCTTTCTTTGAACTTC	1552
<i>S. nux-vomica</i>	GGGTCGGAATTTTGGAA <b>TTTAA</b> ACTCATGTGCGGAAGAAGTAGCCCTTTCTTTGAACTTC	1556
<i>S. thorelii</i>	GGGTCGGAATTTTGGAA <b>TTTAA</b> ACTCATGTGCGGAAGAAGTAGCCCTTTCTTTGAACTTC	1554
<i>S. nux-blanda</i>	GGGTCGGAATTTTGGAA <b>TTTAA</b> ACTCATGTGCGGAAGAAGTAGCCCTTTCTTTGAACTTC	1553
*****		
▽ <i>DraI</i>		
<i>S. lucida</i>	CCAAGAGTTTCTTCGCCCTTTTGGGGGGTGTATAGAAGTCGGATTTGG-ATTTTGATATT	1611
<i>S. nux-vomica</i>	CCAAGAGTTTCTTCGCCCTTTTGGGGGGTGTATAGAAGTCGGATTTGGTATTTTGGTATT	1616
<i>S. thorelii</i>	CCAAGAGTTTCTTCTCCCTTTTGGGGGGTGTATAGAAGTCGGAT <b>TTTA</b> -- <b>AA</b> TTTGGTATT	1612
<i>S. nux-blanda</i>	CCAAGAGTTTCTTCGCCCTTTTGGGGGGTGTATAGAAGTCGGAT <b>TTTA</b> -- <b>AA</b> TTTGGTATT	1611
*****		
▽ <i>DraI</i>		
<i>S. lucida</i>	TGGAATTGTATAACTGATCTGGTGAATCAGCAATGATTCATATTCATTCTGAGACCTTGT	1671
<i>S. nux-vomica</i>	TGGAAT <b>TTTAA</b> ACTGATCTGGTGAATCAGCAATGATTCATATTCATTCTGAGACCTTGT	1676
<i>S. thorelii</i>	TGGA-TATTATAACTGATCTGGTGAATCAGCAATGATTCATATTCATTCTGAGACCTTGT	1671
<i>S. nux-blanda</i>	TGGA-TATTATAACTGATCTGGTGAATCAGCAATGATTCATATTCATTCTGAGACCTTGT	1670
**** * * * *****		
<i>S. lucida</i>	AAATGGATTTAAGCCTAAATGAAATGATGAAGAGATAACAAAAGTTTCTACTATTCTG	1731
<i>S. nux-vomica</i>	AAATGGATTTAAGCCTAAATGAAATGATGAAGAGATAACAAAAGTTTCTACTATTCTG	1736
<i>S. thorelii</i>	AAATGGATTTAAGCCTAAATGAAATGATGAAGAGATAACAAAAGTTTCTACTATTCTG	1731
<i>S. nux-blanda</i>	AAATGGATTTAAGCCTAAATGAAATGATGAAGAGATAACAAAAGTTTCTACTATTCTG	1730
*****		
<i>S. lucida</i>	AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCTTTCTAAACTA	1791
<i>S. nux-vomica</i>	AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCTTTCTAAATA	1796
<i>S. thorelii</i>	AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCTTTCTAAATTA	1791
<i>S. nux-blanda</i>	AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCTTTTGAATA	1790
*****		
<i>S. lucida</i>	AAGTTTAAATGAGTTATCA	1810
<i>S. nux-vomica</i>	AAGTCTAAAAAAGGAAAAA	1815
<i>S. thorelii</i>	AAGTCTAAAAAAGAAA	1810
<i>S. nux-blanda</i>	AAGTCTTAAAAAGGAAACA	1809
**** * * * * *		

**Figure 42** The nucleotide sequence alignment of *matK* gene from four *Strychnos* species (Bolder letters indicated the Recognition site, ▽ indicated the position of restriction site)



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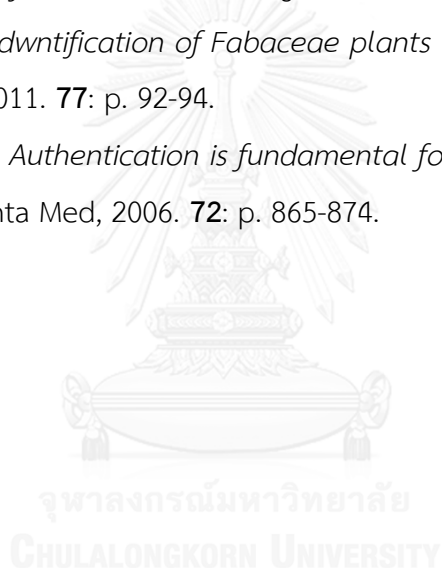
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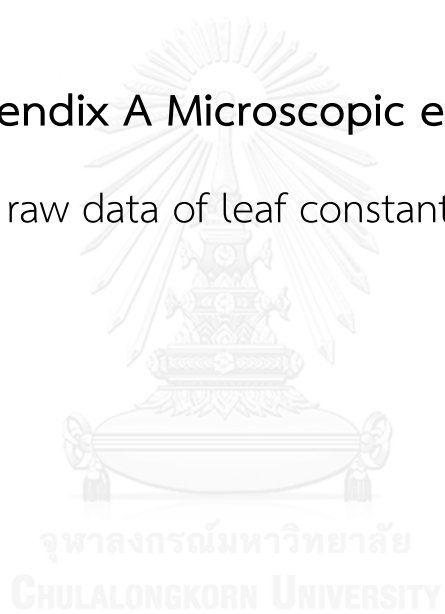


## APPENDICES



## Appendix A Microscopic evaluation

The raw data of leaf constant numbers



**Table 16** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. thorelii*, collected from Chachoengsao province 1.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal ell	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	1132	152	11.84	16	4	1704	586.85	16.50
2	1168	144	10.98	19	4.75	1724	580.05	15.50
3	1116	144	11.43	19	4.75	1580	632.91	16.00
4	1140	148	11.49	16	4	1560	641.03	18.00
5	1172	176	13.06	15	3.75	1512	661.38	16.75
6	1124	156	12.19	19	4.75	1500	666.67	18.25
7	1160	156	11.85	16	4	1584	631.31	15.25
8	1088	156	12.54	14	3.5	1600	625.00	16.25
9	1180	144	10.88	16	4	1624	615.76	17.75
10	1196	136	10.21	21	5.25	1640	609.76	17.00
11	1144	156	12.00	18	4.5	1556	642.67	18.75
12	1116	144	11.43	17	4.25	1644	608.27	16.00
13	1176	164	12.24	15	3.75	1608	621.89	16.50
14	1160	140	10.77	17	4.25	1536	651.04	16.25
15	1188	176	12.90	18	4.5	1676	596.66	16.00
16	1232	144	10.47	17	4.25	1588	629.72	15.00
17	1240	168	11.93	18	4.5	1596	626.57	16.00
18	1200	152	11.24	20	5	1496	668.45	17.00
19	1156	160	12.16	19	4.75	1584	631.31	17.50
20	1168	152	11.52	18	4.5	1548	645.99	15.50
21	1240	164	11.68	17	4.25	1492	670.24	16.00
22	1204	164	11.99	21	5.25	1636	611.25	17.75
23	1124	144	11.36	18	4.5	1596	626.57	15.50
24	1144	152	11.73	19	4.75	1572	636.13	15.00
25	1148	156	11.96	21	5.25	1532	652.74	15.00
26	1144	164	12.54	16	4	1504	664.89	15.25
27	1148	148	11.42	20	5	1716	582.75	14.50
28	1112	156	12.30	18	4.5	1628	614.25	15.00
29	1124	152	11.91	16	4	1652	605.33	14.75
30	1216	160	11.63	15	3.75	1572	636.13	15.00
	Mean	154.27	11.72	Mean	4.41	Mean	614.40	16.18
	S.D.	9.95	0.66	S.D.	0.48	S.D.	13.85	1.14

**Table 17** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. thorelii*, collected from Chachoengsao province 2.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	1172	180	13.31	17	4.25	1556	642.67	14.00
2	1300	160	10.96	19	4.75	1624	615.76	14.50
3	1204	152	11.21	19	4.75	1692	591.02	14.75
4	1228	172	12.29	18	4.5	1504	664.89	14.25
5	1320	148	10.08	17	4.25	1580	632.91	13.50
6	1200	160	11.76	18	4.5	1648	606.80	15.25
7	1208	152	11.18	19	4.75	1504	664.89	13.75
8	1296	164	11.23	18	4.5	1536	651.04	16.75
9	1316	172	11.56	19	4.75	1636	611.25	14.00
10	1384	156	10.13	19	4.75	1716	582.75	14.50
11	1388	180	11.48	18	4.5	1660	602.41	14.00
12	1244	164	11.65	19	4.75	1696	589.62	14.75
13	1284	172	11.81	17	4.25	1604	623.44	13.50
14	1348	148	9.89	18	4.5	1708	585.48	13.25
15	1264	152	10.73	20	5	1492	670.24	15.50
16	1248	180	12.61	16	4	1576	634.52	13.50
17	1348	176	11.55	16	4	1700	588.24	13.75
18	1244	160	11.40	17	4.25	1684	593.82	14.25
19	1280	164	11.36	17	4.25	1600	625.00	15.00
20	1320	180	12.00	18	4.5	1488	672.04	13.50
21	1340	152	10.19	19	4.75	1612	620.35	14.25
22	1276	168	11.63	18	4.5	1512	661.38	14.75
23	1344	152	10.16	17	4.25	1520	657.89	16.25
24	1320	164	11.05	20	5	1480	675.68	17.00
25	1336	152	10.22	15	3.75	1652	605.33	17.25
26	1356	184	11.95	16	4	1624	615.76	13.50
27	1352	168	11.05	20	5	1512	661.38	14.00
28	1364	156	10.26	17	4.25	1672	598.09	13.25
29	1268	176	12.19	17	4.25	1636	611.25	15.75
30	1292	184	12.47	15	3.75	1648	606.80	13.50
	Mean	164.93	11.51	Mean	4.44	Mean	606.77	14.57
	S.D.	11.54	0.85	S.D.	0.48	S.D.	13.58	1.13

**Table 18** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. thorelii*, collected from Chonburi province.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index (sq.mm)	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	1268	176	12.19	15	3.75	1680	595.24	17.25
2	1300	148	10.22	19	4.75	1656	603.86	15.75
3	1232	144	10.47	22	5.5	1736	576.04	15.00
4	1208	164	11.95	18	4.5	1676	596.66	16.75
5	1204	180	13.01	17	4.25	1612	620.35	17.25
6	1220	184	13.11	18	4.5	1544	647.67	15.75
7	1208	160	11.70	19	4.75	1688	592.42	15.00
8	1240	188	13.17	20	5	1600	625.00	16.25
9	1300	172	11.68	17	4.25	1612	620.35	15.50
10	1216	164	11.88	22	5.5	1648	606.80	16.00
11	1196	180	13.08	19	4.75	1588	629.72	15.75
12	1196	156	11.54	18	4.5	1672	598.09	16.25
13	1168	176	13.10	19	4.75	1704	586.85	13.75
14	1216	184	13.14	23	5.75	1684	593.82	15.25
15	1252	188	13.06	16	4	1616	618.81	16.50
16	1324	152	10.30	25	5.25	1592	628.14	17.50
17	1264	148	10.48	19	4.75	1736	576.04	15.50
18	1252	164	11.58	15	3.75	1608	621.89	16.00
19	1248	168	11.86	18	4.5	1716	582.75	14.00
20	1256	160	11.30	18	4.5	1740	574.71	17.00
21	1140	156	12.04	17	4.25	1700	588.24	16.50
22	1244	168	11.90	20	5	1708	585.48	17.75
23	1204	176	12.75	16	4	1580	632.91	15.00
24	1288	152	10.56	23	5.75	1676	596.66	15.25
25	1180	164	12.20	18	4.5	1648	606.80	14.75
26	1188	148	11.08	22	5.5	1676	596.66	15.25
27	1104	176	13.75	23	5.75	1648	606.80	14.50
28	1240	180	12.68	18	4.5	1704	586.85	15.00
29	1212	152	11.14	19	4.75	1604	623.44	13.25
30	1188	172	12.65	17	4.25	1708	585.48	14.25
	Mean	166.67	11.98	Mean	4.42	Mean	598.83	15.65
	S.D.	13.13	0.99	S.D.	0.58	S.D.	13.58	1.13

**Table 19** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. lucida*, collected from Bangkok province.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	2784	160	5.43	30	7.5	2464	405.84	15.00
2	2804	184	6.16	28	7	2412	414.59	16.25
3	2988	184	5.80	26	6.5	2524	396.20	16.75
4	2860	184	6.04	27	6.75	2472	404.53	17.50
5	2776	196	6.59	23	5.75	2496	400.64	17.25
6	2796	176	5.92	24	6	2432	411.18	18.50
7	2716	152	5.30	28	7	2492	401.28	17.75
8	2688	168	5.88	24	6	2212	452.08	15.75
9	2832	168	5.60	27	6.75	2432	411.18	20.25
10	2676	148	5.24	25	6.25	2324	430.29	16.25
11	2656	176	6.21	26	6.5	2516	397.46	16.00
12	2840	152	5.08	23	5.75	2508	398.72	13.75
13	2704	152	5.32	29	7.25	2340	427.35	17.50
14	2732	164	5.66	30	7.5	2444	409.17	17.25
15	2640	140	5.04	30	7.5	2192	456.20	18.00
16	2876	180	5.89	24	6	2356	424.45	18.50
17	2608	144	5.23	26	6.5	2212	452.08	17.00
18	2592	144	5.26	25	6.25	2348	425.89	15.25
19	2740	148	5.12	21	5.25	2200	454.55	17.00
20	2628	164	5.87	26	6.5	2360	423.73	16.25
21	2696	180	6.26	22	5.5	2196	455.37	17.50
22	2860	184	6.04	24	6	2312	432.53	14.50
23	2816	176	5.88	23	5.75	2236	447.23	16.75
24	2756	160	5.49	23	5.75	2376	420.88	14.25
25	2700	176	6.12	29	7.25	2200	454.55	15.00
26	2892	164	5.37	25	6.25	2388	418.76	15.50
27	2856	180	5.93	28	7	2296	435.54	15.00
28	2956	192	6.10	33	8.25	2276	439.37	14.75
29	2688	152	5.35	29	7.25	2224	449.64	14.00
30	2888	176	5.74	22	5.5	2268	440.92	16.50
	Mean	167.47	6.77	Mean	6.50	Mean	419.32	16.42
	S.D.	15.61	0.73	S.D.	0.73	S.D.	8.32	1.51

**Table 20** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. lucida*, collected from Pathum Thani province.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	1888	164	7.99	31	7.75	2200	454.55	15.75
2	1804	180	9.07	23	5.75	2208	452.90	19.50
3	1892	160	7.80	25	6.25	2328	429.55	17.25
4	2204	184	7.71	28	7	2100	476.19	18.50
5	1876	184	8.93	22	5.5	2100	476.19	17.50
6	1844	160	7.98	24	6	2212	452.08	18.25
7	2188	172	7.29	26	6.5	2504	399.36	15.00
8	2212	164	6.90	27	6.75	2256	443.26	15.00
9	2008	196	8.89	28	7	2512	398.09	17.50
10	1872	184	8.95	29	7.25	2132	469.04	16.50
11	1864	188	9.16	23	5.75	2448	408.50	14.50
12	2156	188	8.02	23	5.75	2108	474.38	14.00
13	2196	196	8.19	30	7.5	2164	462.11	15.75
14	2120	196	8.46	26	6.5	2184	457.88	16.25
15	2184	192	8.08	27	6.75	2120	471.70	15.50
16	1744	180	9.36	29	7.25	2144	466.42	16.75
17	1780	156	8.06	30	7.5	2112	473.48	15.25
18	1792	152	7.82	32	8	2148	465.55	14.25
19	1824	160	8.06	24	6	2244	445.63	20.00
20	1736	176	9.21	30	7.5	2128	469.92	18.75
21	1756	156	8.16	26	6.5	2132	469.04	17.25
22	2052	184	8.23	36	9	2296	435.54	18.25
23	2296	176	7.12	25	6.25	2268	440.92	15.25
24	2220	192	7.96	24	6	2396	417.36	16.50
25	2208	184	7.69	29	7.25	2412	414.59	16.00
26	2008	168	7.72	27	6.75	2304	434.03	16.25
27	2232	192	7.92	28	7	2468	405.19	19.00
28	2204	192	8.01	29	7.25	2492	401.28	18.50
29	2184	196	8.24	26	6.5	2320	431.03	15.00
30	1768	164	8.49	28	7	2352	425.17	16.00
	Mean	177.87	7.52	Mean	6.79	Mean	425.75	16.66
	S.D.	14.16	0.64	S.D.	0.78	S.D.	8.10	1.63



**Table 21** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. lucida*, collected from Nonthaburi province.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	2104	156	6.90	26	6.5	2088	478.93	13.50
2	2032	164	7.47	28	7	2036	491.16	15.50
3	2080	176	7.80	22	5.5	2456	407.17	15.00
4	2268	168	6.90	27	6.75	2452	407.83	17.75
5	2000	164	7.58	28	7	2840	352.11	15.75
6	2044	172	7.76	30	7.5	2076	481.70	16.50
7	2396	180	6.99	25	6.25	2256	443.26	14.50
8	2140	164	7.12	30	7.5	2560	390.63	17.50
9	2240	168	6.98	25	6.25	2380	420.17	18.75
10	2220	176	7.35	27	6.75	1940	515.46	19.00
11	1816	184	9.20	24	6	2096	477.10	14.75
12	2064	172	7.69	30	7.5	2392	418.06	16.25
13	2212	160	6.75	26	6.5	2656	376.51	18.00
14	2196	172	7.26	28	7	2324	430.29	15.75
15	2160	176	7.53	25	6.25	2280	438.60	19.00
16	2108	168	7.38	29	7.25	2356	424.45	14.75
17	2064	152	6.86	26	6.5	2732	366.03	15.00
18	1972	168	7.85	24	6	2340	427.35	16.75
19	2136	152	6.64	25	6.25	2476	403.88	19.75
20	2060	192	8.53	23	5.75	2688	372.02	19.25
21	2192	160	6.80	31	7.75	2852	350.63	17.75
22	2168	164	7.03	29	7.25	2556	391.24	14.00
23	2252	160	6.63	32	8	2396	417.36	12.00
24	2180	188	7.94	28	7	1944	514.40	12.75
25	2112	184	8.01	32	8	2336	428.08	18.50
26	2040	164	7.44	27	6.75	2612	382.85	13.75
27	2072	168	7.50	26	6.5	2524	396.20	14.75
28	2132	184	7.94	25	6.25	2720	367.65	19.25
29	1984	188	8.66	32	8	1960	510.20	18.00
30	2128	168	7.32	23	5.75	2128	469.92	12.25
	Mean	170.40	7.35	Mean	6.78	Mean	422.34	17.78
	S.D.	10.64	0.50	S.D.	0.69	S.D.	8.04	1.95

**Table 22** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. nux-vomica*, sample collected from Pathum Thani province.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	2320	220	8.66	26	6.5	1424	702.25	20.50
2	2160	204	8.63	30	7.5	1504	664.89	22.50
3	2140	232	9.78	29	7.25	1492	670.24	19.75
4	2144	212	9.00	36	9	1428	700.28	16.50
5	2052	196	8.72	37	9.25	1368	730.99	20.75
6	2288	212	8.48	30	7.5	1476	677.51	21.00
7	1996	200	9.11	34	8.5	1392	718.39	18.00
8	2052	204	9.04	36	9	1500	666.67	18.50
9	2056	196	8.70	32	8	1248	801.28	21.00
10	2340	212	8.31	30	7.5	1508	663.13	22.75
11	2368	224	8.64	25	6.25	1272	786.16	22.25
12	2204	192	8.01	31	7.75	1376	726.74	18.75
13	2284	208	8.35	31	7.75	1320	757.58	16.75
14	2240	196	8.05	28	7	1460	684.93	17.50
15	2108	216	9.29	33	8.25	1432	698.32	16.75
16	2292	224	8.90	30	7.5	1364	733.14	18.50
17	2196	192	8.04	37	9.25	1316	759.88	18.00
18	2440	208	7.85	30	7.5	1456	686.81	21.50
19	2336	224	8.75	32	8	1432	698.32	19.00
20	2476	204	7.61	31	7.75	1368	730.99	17.25
21	2388	220	8.44	30	7.5	1360	735.29	17.00
22	2396	240	9.10	32	8	1380	724.64	17.75
23	2324	224	8.79	32	8	1424	702.25	16.50
24	2296	228	9.03	30	7.5	1504	664.89	19.00
25	2160	216	9.09	30	7.5	1432	698.32	20.00
26	2220	244	9.90	35	8.75	1316	759.88	20.25
27	2240	216	8.79	35	8.75	1368	730.99	19.00
28	2388	220	8.44	33	8.25	1424	702.25	20.50
29	2300	228	9.02	35	8.75	1388	720.46	18.75
30	2484	236	8.68	34	8.5	1508	663.13	20.25
	Mean	214.93	8.71	Mean	7.98	Mean	709.16	19.22
	S.D.	14.13	0.51	S.D.	0.75	S.D.	15.30	1.84

**Table 23** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. nux-vomica*, collected from Khon Kaen province.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	2148	188	8.05	40	10	1420	704.23	16.25
2	2272	192	7.79	36	9	1464	683.06	15.25
3	2100	200	8.70	34	8.5	1368	730.99	16.00
4	2108	220	9.45	40	10	1452	688.71	15.00
5	2132	200	8.58	34	8.5	1440	694.44	14.75
6	2212	208	8.60	39	9.75	1432	698.32	18.25
7	2184	236	9.75	34	8.5	1460	684.93	18.25
8	2172	188	7.97	38	9.5	1404	712.25	18.75
9	2172	204	8.59	37	9.25	1444	692.52	15.50
10	2128	208	8.90	37	9.25	1420	704.23	20.75
11	2100	224	9.64	36	9	1340	746.27	16.50
12	2048	212	9.38	35	8.75	1404	712.25	17.00
13	2092	204	8.89	40	10	1348	741.84	15.25
14	2016	196	8.86	37	9.25	1396	716.33	18.50
15	2096	200	8.71	33	8.25	1396	716.33	17.25
16	2136	208	8.87	39	9.75	1352	739.64	17.5
17	2160	200	8.47	40	10	1340	746.27	15.00
18	2100	196	8.54	42	10.5	1364	733.14	16.25
19	2060	212	9.33	33	8.25	1284	778.82	15.25
20	2112	188	8.17	38	9.5	1228	814.33	18.25
21	2052	208	9.20	41	10.25	1412	708.22	17.25
22	2052	188	8.39	31	7.75	1368	730.99	20.00
23	2056	196	8.70	38	9.5	1292	773.99	16.75
24	2108	200	8.67	36	9	1352	739.64	15.50
25	2140	196	8.39	37	9.25	1392	718.39	16.50
26	2128	196	8.43	37	9.25	1400	714.29	20.25
27	2052	196	8.72	43	10.75	1320	757.58	18.00
28	2096	212	9.19	39	9.75	1368	730.99	18.75
29	2188	200	8.38	40	10	1372	728.86	16.00
30	2196	212	8.80	39	9.75	1324	755.29	17.00
	Mean	202.93	8.74	Mean	9.36	Mean	724.61	17.06
	S.D.	11.16	0.48	S.D.	0.72	S.D.	17.79	1.65

**Table 24** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. nux-vomica*, collected from Song khla province.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	2092	180	7.92	39	8.75	1252	798.72	19.50
2	2156	200	8.49	36	9	1244	803.86	20.25
3	2184	196	8.24	36	9	1252	798.72	20.00
4	2076	196	8.63	44	11	1280	781.25	17.25
5	2032	208	9.29	38	9.5	1272	786.16	16.25
6	2048	200	8.90	39	9.75	1268	788.64	19.00
7	2096	180	7.91	37	9.25	1252	798.72	20.75
8	2156	192	8.18	35	8.75	1240	806.45	21.50
9	2120	208	8.93	32	8	1260	793.65	14.75
10	2108	232	9.91	38	9.5	1308	764.53	17.75
11	2036	212	9.43	36	9	1288	776.40	14.50
12	2124	220	9.39	40	10	1320	757.58	15.75
13	2120	196	8.46	38	9.5	1372	728.86	15.25
14	2116	188	8.16	39	9.75	1224	816.99	18.25
15	2044	208	9.24	44	11	1364	733.14	17.25
16	2084	184	8.11	35	8.75	1352	739.64	15.75
17	2168	192	8.14	38	9.5	1280	781.25	21.50
18	2156	200	8.49	34	8.5	1316	759.88	16.75
19	2212	196	8.14	41	10.25	1280	781.25	17.50
20	2168	188	7.98	39	9.75	1264	791.14	18.25
21	2160	184	7.85	43	10.75	1236	809.06	14.25
22	2116	196	8.48	40	10	1296	771.60	15.00
23	2124	204	8.76	36	9	1336	748.50	18.50
24	2120	192	8.30	33	8.25	1408	710.23	16.50
25	2080	188	8.29	38	9.5	1260	793.65	16.25
26	2052	180	8.06	33	8.25	1268	788.64	14.75
27	2052	200	8.88	37	9.25	1284	778.82	19.75
28	2092	192	8.41	42	10.5	1224	816.99	19.50
29	2020	196	8.84	43	10.75	1224	816.99	16.00
30	2052	184	8.23	35	8.75	1284	778.82	18.75
	Mean	196.40	8.56	Mean	9.45	Mean	710.23	17.78
	S.D.	11.95	0.52	S.D.	0.82	S.D.	18.04	1.95

**Table 25** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. nux-blanda*, collected from Chiang Mai province 1.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	1296	236	15.40	45	11.25	856	1168.22	18.00
2	1348	252	15.75	49	12.25	952	1050.42	18.00
3	1332	224	14.40	51	12.75	852	1173.71	17.75
4	1396	244	14.88	48	12	844	1184.83	17.00
5	1308	244	15.72	50	12.5	884	1131.22	17.00
6	1264	264	17.28	45	11.25	856	1168.22	15.75
7	1364	240	14.96	52	13	868	1152.07	13.75
8	1264	236	15.73	46	11.5	868	1152.07	15.00
9	1380	224	13.97	45	11.25	852	1173.71	15.50
10	1356	220	13.96	53	13.25	888	1126.13	16.75
11	1284	240	15.75	49	12.25	852	1173.71	18.50
12	1344	268	16.63	52	13	840	1190.48	17.75
13	1388	240	14.74	50	12.5	912	1096.49	15.75
14	1336	248	15.66	44	11	860	1162.79	16.75
15	1292	212	14.10	48	12	864	1157.41	19.25
16	1404	252	15.22	47	11.75	844	1184.83	14.75
17	1324	252	15.99	43	10.75	872	1146.79	15.50
18	1272	220	14.75	45	11.25	912	1096.49	15.50
19	1396	216	13.40	51	12.75	844	1184.83	19.50
20	1328	224	14.43	55	13.75	888	1126.13	14.25
21	1312	240	15.46	47	11.75	916	1091.70	16.25
22	1276	244	16.05	50	12.5	880	1136.36	14.50
23	1288	236	15.49	48	12	900	1111.11	15.50
24	1356	252	15.67	49	12.25	884	1131.22	15.00
25	1300	236	15.36	51	12.75	828	1207.73	12.50
26	1316	236	15.21	43	10.75	908	1101.32	15.25
27	1256	248	16.49	49	12.25	852	1173.71	17.50
28	1388	232	14.32	48	12	884	1131.22	16.75
29	1408	252	15.18	53	13.25	832	1201.92	18.25
30	1372	248	15.31	43	10.75	876	1141.55	17.00
	Mean	239.33	15.24	Mean	12.08	Mean	1130.95	16.35
	S.D.	13.62	0.86	S.D.	0.81	S.D.	25.82	1.66

**Table 26** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. nux-blanda*, collected from Chiang Mai province 2.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	1200	288	19.35	55	13.75	980	1020.41	17.50
2	1284	256	16.62	53	13.25	924	1082.25	18.75
3	1264	256	16.84	57	14.25	960	1041.67	15.00
4	1332	260	16.33	49	12.25	996	1004.02	16.75
5	1404	248	15.01	52	13	952	1050.42	17.75
6	1344	236	14.94	55	13.75	1000	1000.00	16.25
7	1300	252	16.24	51	12.75	908	1101.32	17.50
8	1252	240	16.09	55	13.75	1000	1000.00	17.25
9	1304	268	17.05	47	11.75	984	1016.26	17.50
10	1284	216	14.40	53	13.25	1004	996.02	18.50
11	1276	268	17.36	52	13	960	1041.67	15.75
12	1304	228	14.88	50	12.5	956	1046.03	18.75
13	1332	276	17.16	49	12.25	900	1111.11	17.50
14	1236	264	17.60	53	13.25	916	1091.70	16.00
15	1332	268	16.75	46	11.5	984	1016.26	17.75
16	1328	220	14.21	51	12.75	956	1046.03	17.50
17	1360	264	16.26	53	13.25	948	1054.85	15.50
18	1260	252	16.67	48	12	992	1008.06	14.25
19	1280	284	18.16	46	11.5	980	1020.41	16.25
20	1328	276	17.21	46	11.5	924	1082.25	17.25
21	1320	264	16.67	50	12.5	956	1046.03	14.75
22	1372	228	14.25	48	12	972	1028.81	17.25
23	1308	260	16.58	47	11.75	916	1091.70	19.00
24	1300	256	16.45	51	12.75	940	1063.83	17.75
25	1320	256	16.24	45	11.25	956	1046.03	17.00
26	1368	264	16.18	49	12.25	988	1012.15	16.25
27	1288	256	16.58	46	11.5	980	1020.41	17.50
28	1328	276	17.21	47	11.75	880	1136.36	16.00
29	1348	280	17.20	51	12.75	864	1157.41	18.75
30	1300	260	16.67	48	12	908	1101.32	14.75
	Mean	257.33	16.44	Mean	12.53	Mean	1063.64	16.92
	S.D.	18.08	1.14	S.D.	0.81	S.D.	21.57	1.29

**Table 27** Stomatal number, stomatal index, palisade ratio, epidermal cell number, epidermal cell area and veinlet termination number of *S. nux-blanda*, collected from Chiang Mai province 3.

File	Stomatal cell			Palisade cell		Epidermal cell		Veinlet termination number (sq.mm)
	Number of epidermal cells (sq.mm)	Stomatal number (sq.mm)	Stomatal index	Number of beneath 4 epidermal cells	Palisade ratio	Number of epidermal cells (sq.mm)	epidermal cell area (sq.µm)	
1	1352	260	16.13	50	12.5	908	1101.32	15.25
2	1316	240	15.42	52	13	944	1059.32	14.75
3	1308	232	15.06	51	12.75	980	1020.41	14.25
4	1304	224	14.66	47	11.75	936	1068.38	15.00
5	1292	236	15.45	54	13.5	908	1101.32	14.75
6	1364	268	16.42	48	12	876	1141.55	14.50
7	1328	244	15.52	47	11.75	868	1152.07	15.25
8	1296	252	16.28	45	11.25	864	1157.41	15.75
9	1320	256	16.24	54	13.5	876	1141.55	16.75
10	1332	280	17.37	44	11	848	1179.25	17.50
11	1284	264	17.05	50	12.5	880	1136.36	14.00
12	1280	272	17.53	48	12	848	1179.25	16.25
13	1292	228	15.00	57	14.25	896	1116.07	14.50
14	1340	220	14.10	50	12.5	872	1146.79	13.50
15	1360	240	15.00	46	11.5	888	1126.13	15.75
16	1284	276	17.69	44	11	884	1131.22	17.00
17	1356	224	14.18	49	12.25	856	1168.22	14.25
18	1300	284	17.93	48	12	960	1041.67	15.00
19	1316	256	16.28	51	12.75	852	1173.71	17.50
20	1340	284	17.49	46	11.5	844	1184.83	15.50
21	1320	272	17.09	47	11.75	860	1162.79	16.00
22	1264	272	17.71	52	13	916	1091.70	14.75
23	1316	224	14.55	44	11	844	1184.83	15.00
24	1284	244	15.97	50	12.5	936	1068.38	17.75
25	1292	280	17.81	49	12.25	872	1146.79	15.75
26	1340	244	15.40	52	13	896	1116.07	14.75
27	1300	276	17.51	45	11.25	880	1136.36	16.00
28	1292	256	16.54	45	11.25	844	1184.83	14.00
29	1296	268	17.14	54	13.5	908	1101.32	15.50
30	1308	272	17.22	51	12.75	928	1077.59	18.25
	Mean	254.93	16.26	Mean	12.27	Mean	1113.36	15.49
	S.D.	20.31	1.18	S.D.	0.85	S.D.	26.82	1.21

## Appendix B Molecular evaluation

(The ITS, *rbcL* and *matK* sequence and the multiple sequence alignment of four *Strychnos* species collected from different locations of Thailand)



## The ITS sequence of *S. thorelii*, *S. nux-vomica*, *S. lucida* and *S. nux-blanda*

### >ITS-St1

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### >ITS-St2

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### >ITS-St4

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The multiple sequence alignment of ITS region

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ITS-Sv1      TTTCTCCGGCTATTGATATGCTTAAACTCAGCG--GGTA----ATCCCACCTGACCTG  54
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ITS-Sv3      TTTCTCCGGCTATTGATATGCTTAAACTCAGCG--GGTATGGATCCCCTGACCTG  58
ITS-S11      TTTCTCCGGCTATTGATATGCTTAAACTCAGCG-GTGT---GTCCCACCTGACCTG  55
ITS-S12      TTTCTCCG-CTTATTGATATGCTTAA-CTCAACG-G-GTA---GTCCCACCTGACCTG  52
ITS-S13      TTTCTCCGGCTATTGATATGCTTAAACTCAACG-G-GTA---GTCCCACCTGACCTG  54
ITS-Sb2      TTTCTCCGGC-TAATGATATGCTTAAACTCAGCG--GGTTT-AGTCCCCTGACCTG  55
ITS-Sb3      TTTCTCCGGC-TA-TGATATGCTTAAACTCAGCG--GGT---AGTCCCCTGACCTG  52
ITS-Sb1      TTTCTCCGGCTATTGATATGCTTAAACTCAGCG--GGTTTT-AGTCACGCTGAACG  57
ITS-Sb4      TTTCTCCGGC-TATTGATATGCTTAAACTCAGCG--GGTATT-TGTACCCTGACCTG  56
ITS-St1      TTTCTCCGAATAAT-GTTATGCTTAAACTCAA-C-GGGTA----GTACCCTGACCTG  53
ITS-St4      TTTCTCCGGCTA-T-GATATGCTTAAACTCAAGC-GGGTA----ATCCCACCTGACCTG  53
ITS-St2      TTTCTCCGGCTAT-GATATGCTTAAACTCAGCT-GGGAA----GTCCCACCTGACCTG  54
ITS-St3      TTTCTCCGACTTATTGATATGCTTAAACTCAGC--GGGAA----GTCCCACCTGACCTG  54
          **** * *          * ***** **          *          * * ***** **

ITS-Sv4      GGGTCGC-GGTCGTGGCCGAGCGTCTCGGGACGCCTGG-CGGCAGGGTCGGGCAGTCC  114
ITS-Sv5      GGGTCGC-GGTCGTGGCCGAGCGTCTCGGGACGCCTGG-CGGCAGGGTCGGGCAGTCC  113
ITS-Sv1      GGGTCGC-GGTCGTGGCCGAGCGTCTCGGGACGCCTGG-CGGCAGGGTCGGGCAGTCC  112
ITS-Sv2      GGGTCGC-GGTCGTGGCCGAGCGTCTCGGGACGCCTGG-CGGCAGGGTCGGGCAGTCC  112
ITS-Sv3      GGGTCGC-GGTCGTGGCCGAGCGTCTCGGGACGCCTGG-CGGCAGGGTCGGGCAGTCC  116
ITS-S11      GGGTCGC-GGTCGTGGCCGAGCGTCTCGGGACGCCTGG-CGGCAGGGTCGGGCAGTCC  113
ITS-S12      GGGTCGC-GGTCGTGGCCGAGCGTCTCGGGACGCCTGG-CGGCAGGGTCGGGCAGTCC  110
ITS-S13      GGTTTCGC-GGTCGTGGCCGAGCGTCTCGGGACGCCTGG-CGGCAGGGTCGGGCAGTCC  112
ITS-Sb2      GGGTCGCTGGTCGTGGCCGAGCGTCCACGGGACGCCTGGGCAGAAGGGTCACGGCAGTCC  115
ITS-Sb3      GGGTCGC-GGTCGTGGCCGAGCGTCCACGGGACGCCTGG-CAGAAGGGTCACGGCAGTCC  110
ITS-Sb1      GGGTCGC-GGTCGTGGCCGAGCGTCCACGGGACGCCTGG-CAGAAGGGTCACGGCAGTCC  115
ITS-Sb4      GGGTCGC-GGTCGTGGCCGAGCGTCCACGGGACGCCTGG-CAGAAGGGTCACGGCAGTCC  114
ITS-St1      GGGTCGC-GGTCGTAGCCGAGCATCAAAGAGATGC-TGG-CTTCAGGGTCATGGCAGTCC  110
ITS-St4      GGGTCGC-GGTCGTAGCCGAGCATCAAAGAGATGC-TGG-CTTCAGGGTCATGGCAGTCC  110
ITS-St2      GGGTCGC-GGTCGTAGCCGAGCATCAAAGAGATGC-TGG-CTTCAGGGTCATGGCAGTCC  111
ITS-St3      GGGTCGC-GGTCGTAGCCGAGCATCAAAGAGATGC-TGG-CTTCAGGGTCATGGCAGTCC  111
          ** **** ***** ***** **   * * * * * * *   ***** ***** **

ITS-Sv4      GCTCGAGCGACGGGCTCCGACACGACAGCTATGCGAGTTGAGGCATTCAACCACCACTTG  174
ITS-Sv5      GCTCGAGCGACGGGCTCCGACACGACAGCTATGCGAGTTGAGGCATTCAACCACCACTTG  173
ITS-Sv1      GCTCGAGCGACGGGCTCCGACACGACAGCTATGCGAGTTGAGGCATTCAACCACCACTTG  172
ITS-Sv2      GCTCGAGCGACGGGCTCCGACACGACAGCTATGCGAGTTGAGGCATTCAACCACCACTTG  172
ITS-Sv3      GCTCGAGCGACGGGCTCCGACACGACAGCTATGCGAGTTGAGGCATTCAACCACCACTTG  176
ITS-S11      GCTCGAGCGACGGGTTCCGGGACGACAGCTATGCGAGTTGAGGCATTCAACCACCACTTG  173
ITS-S12      GCTCGAGCGACGGGTTCCGGGACGACAGCTATGCGAGTTGAGGCATTCAACCACCACTTG  170
ITS-S13      GCTCGAGCGACGGGTTCCGGGACGACAGCTATGCGAGTTGAGGCATTCAACCACCACTTG  172
ITS-Sb2      GCTCGAACGACGGGTTCCAACACGACAACACTATGCGAGTTGAGGCATTCAACCACCACTTG  175
ITS-Sb3      GCTCGAACGACGGGTTCCAACACGACAACACTATGCGAGTTGAGGCATTCAACCACCACTTG  170
ITS-Sb1      GCTCGAACGACGGGTTCCAACACGACAACACTATGCGAGTTGAGGCATTCAACCACCACTTG  175
ITS-Sb4      GCTCGAACGACGGGTTCCAACACGACAACACTATGCGAGTTGAGGCATTCAACCACCACTTG  174
ITS-St1      ACACGAGCAACGGGTTTTACACGACAGATATGCGAGTTGAGGCATTCAACCACCACTTG  170
ITS-St4      ACACGAGCAACGGGTTTTACACGACAGATATGCGAGTTGAGGCATTCAACCACCACTTG  170
ITS-St2      ACACGAGCAACGGGTTTTACACGACAGATATGCGAGTTGAGGCATTCAACCACCACTTG  171
ITS-St3      ACACGAGCAACGGGTTTTACACGACAGATATGCGAGTTGAGGCATTCAACCACCACTTG  171
          * ** * * * * * *   * * * * * * * * * * * * * * * * * * * * * * * *

ITS-Sv4      TCGTGACGCCCGTCGTGAGGGACTCTCATTTAGGCCAACCGCGCGCTAGCACGGGAGGCC  234
ITS-Sv5      TCGTGACGCCCGTCGTGAGGGACTCTCATTTAGGCCAACCGCGCGCTAGCACGGGAGGCC  233
ITS-Sv1      TCGTGACGCCCGTCGTGAGGGACTCTCATTTAGGCCAACCGCGCGCTAGCACGGGAGGCC  232
ITS-Sv2      TCGTGACGCCCGTCGTGAGGGACTCTCATTTAGGCCAACCGCGCGCTAGCACGGGAGGCC  232
ITS-Sv3      TCGTGACGCCCGTCGTGAGGGACTCTCATTTAGGCCAACCGCGCGCTAGCACGGGAGGCC  236
ITS-S11      TCGTGACGCCCGTCGTGAGGGACTCTCATTTAGGCCAACCGCGCGCTAGCACGGGAGGCC  233
ITS-S12      TCGTGACGCCCGTCGTGAGGGACTCTCATTTAGGCCAACCGCGCGCTAGCACGGGAGGCC  230
ITS-S13      TCGTGACGCCCGTCGTGAGGGACTCTCATTTAGGCCAACCGCGCGCTAGCACGGGAGGCC  232

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ITS-Sb2 TCGTGACGCCCATCGGGAGGGACTCTCATTTAGGCCAACCGCACGATAGCACGGGAGGCC 235  
 ITS-Sb3 TCGTGACGCCCATCGGGAGGGACTCTCATTTAGGCCAACCGCACGATAGCACGGGAGGCC 230  
 ITS-Sb1 TCGTGACGCCCATCGGGAGGGACTCTCATTTAGGCCAACCGCACGATAGCACGGGAGGCC 235  
 ITS-Sb4 TCGTGACGCCCATCGGGAGGGACTCTCATTTAGGCCAACCGCACGATAGCACGGGAGGCC 234  
 ITS-St1 TCGTGACGCCCGTCGGGAGGGACTCTCATTTAGGCCAACCGCACGCTGGCACGGGAGGCC 230  
 ITS-St4 TCGTGACGCCCGTCGGGAGGGACTCTCATTTAGGCCAACCGCACGCTGGCACGGGAGGCC 230  
 ITS-St2 TCGTGACGCCCGTCGGGAGGGACTCTCATTTAGGCCAACCGCACGCTGGCACGGGAGGCC 231  
 ITS-St3 TCGTGACGCCCGTCGGGAGGGACTCTCATTTAGGCCAACCGCACGCTGGCACGGGAGGCC 231  
 \*\*\*\*\* \*\* \* \*\*\*\*\*  
 ITS-Sv4 ATTGTCCGTCCCCTCCCGCACGGCCCGAGAGGGCGTGTGGGGGAGCGACGCGATGCGTG 294  
 ITS-Sv5 ATTGTCCGTCCCCTCCCGCACGGCCCGAGAGGGCGTGTGGGGGAGCGACGCGATGCGTG 293  
 ITS-Sv1 ATTGTCCGTCCCCTCCCGCACGGCCCGAGAGGCGTGTGGGGGAGCGACGCGATGCGTG 292  
 ITS-Sv2 ATTGTCCGTCCCCTCCCGCACGGCCCGAGAGGGCGTGTGGGGGAGCGACGCGATGCGTG 292  
 ITS-Sv3 ATTGTCCGTCCCCTCCCGCACGGCCCGAGAGGGCGTGTGGGGGAGCGACGCGATGCGTG 296  
 ITS-Sl1 ATTATCCGTCCCCTCCCGCACGGCCCGAGAGGGCGTGTGGGGGAGCGACGCGATGCGTG 293  
 ITS-Sl2 ATTATCCGTCCCCTCCCGCACGGCCCGAGAGGGCGTGTGGGGGAGCGACGCGATGCGTG 290  
 ITS-Sl3 ATTATCCGTCCCCTCCCGCACGGCCCGAGAGGGCGTGTGGGGGAGCGACGCGATGCGTG 292  
 ITS-Sb2 ATTATCCGTCCCCCTCGCACGGCCCGAGCAGGCGTGTGGGGGAGAGACGCGATGCGTG 295  
 ITS-Sb3 ATTATCCGTCCCCCTCGCACGGCCCGAGCAGGCGTGTGGGGGAGAGACGCGATGCGTG 290  
 ITS-Sb1 ATTATCCGTCCCCCTCGCACGGCCCGAGCAGGCGTGTGGGGGAGAGACGCGATGCGTG 295  
 ITS-Sb4 ATTATCCGTCCCCCTCGCACGGCCCGAGCAGGCGTGTGGGGGAGAGACGCGATGCGTG 294  
 ITS-St1 AATATCCGTCCCC-CGCACACGGCATAAGTTGGCGTGTAGGGGATCGACGTGATGCGTG 289  
 ITS-St4 AATATCCGTCCCC-CGCACACGGCATAAGTTGGCGTGTAGGGGATCGACGTGATGCGTG 289  
 ITS-St2 AATATCCGTCCCC-CGCACACGGCATAAGTTGGCGTGTAGGGGATCGACGTGATGCGTG 290  
 ITS-St3 AATATCCGTCCCC-CGCACACGGCATAAGTTGGCGTGTAGGGGATCGACGTGATGCGTG 290  
 \* \* \*\*\*\*\* \* \* \*\*\*\*\* \*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\*  
 ITS-Sv4 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 353  
 ITS-Sv5 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 352  
 ITS-Sv1 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 351  
 ITS-Sv2 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 351  
 ITS-Sv3 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 355  
 ITS-Sl1 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 352  
 ITS-Sl2 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 349  
 ITS-Sl3 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 351  
 ITS-Sb2 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 354  
 ITS-Sb3 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 350  
 ITS-Sb1 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 354  
 ITS-Sb4 ACGCCAGGCAGACGTGCCCTCGGCCTAACGGCTTCGGGCGCAACTTGCCTTCAAA-GAC 353  
 ITS-St1 ACGCCAGGCAGACGTGCCCTCGACCTAGTGGCTTCAGGCGCAACTTGCCTTCAAA-GAC 348  
 ITS-St4 ACGCCAGGCAGACGTGCCCTCGACCTAGTGGCTTCAGGCGCAACTTGCCTTCAAA-GAC 348  
 ITS-St2 ACGCCAGGCAGACGTGCCCTCGACCTAGTGGCTTCAGGCGCAACTTGCCTTCAAA-GAC 349  
 ITS-St3 ACGCCAGGCAGACGTGCCCTCGACCTAGTGGCTTCAGGCGCAACTTGCCTTCAAA-GAC 349  
 \*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\*  
 ITS-Sv4 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 413  
 ITS-Sv5 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 412  
 ITS-Sv1 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 411  
 ITS-Sv2 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 411  
 ITS-Sv3 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 415  
 ITS-Sl1 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 412  
 ITS-Sl2 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 409  
 ITS-Sl3 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 411  
 ITS-Sb2 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 414  
 ITS-Sb3 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 410  
 ITS-Sb1 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 414  
 ITS-Sb4 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 413  
 ITS-St1 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 408  
 ITS-St4 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 408  
 ITS-St2 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 409  
 ITS-St3 TCGATGGTTCACGGGATTCTGCAATTCACACCAAGTATCGCAGTTTGTACGTTCTTCAT 409  
 \*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\*

ITS-Sv4 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTTTAGGTA-GATGCCGAT 472  
 ITS-Sv5 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTTTAGGTA-GATGCCGAT 471  
 ITS-Sv1 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTTTAGGTA-GATGCCGAT 470  
 ITS-Sv2 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTTTAGGTA-GATGCCGAT 470  
 ITS-Sv3 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTTTAGGTA-GATGCCGAT 474  
 ITS-S11 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTTTAGGTA-GATGCCGAT 471  
 ITS-S12 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTTTAGGTA-GATGCCGAT 468  
 ITS-S13 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTTTAGGTA-GATGCCGAT 470  
 ITS-Sb2 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTGTAGGTAAGATGCCGAT 474  
 ITS-Sb3 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTGTAGGTA-GATGCCGAT 469  
 ITS-Sb1 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTGTAGGTA-GATGCCGAT 473  
 ITS-Sb4 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCGTTTTAGTGTAGGTA-GATGCCGAT 472  
 ITS-St1 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCATTTTAGTTTAGGTA-GAAGCCGAA 467  
 ITS-St4 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCATTTTAGTTTAGGTA-GAAGCCGAA 467  
 ITS-St2 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCATTTTAGTTTAGGTA-GAAGCCGAA 468  
 ITS-St3 CGATGCGAGAGCCGAGATATCCGTTGCCGAGAGTCATTTTAGTTTAGGTA-GAAGCCGAA 468  
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ITS-Sv4 CGCCCCCGG---GCGCACCGCGAACGGG-GCCGCGAGGGGCGGGCTATCCG-TGAAAGTT 527  
 ITS-Sv5 CGCCCCCGG---GCGCACCGCGAACGGG-GCCGCGAGGGGCGGGCTATCCG-TGAAAGTT 526  
 ITS-Sv1 CGCCCCCGG---GCGCACCGCGAACCTTG-GCCGCGAGGGGCGGGCTATCCG-TGAAAGTT 525  
 ITS-Sv2 CGCCCCCGG---GCGCACCGCGAACGGG-GCCGCGAGGGGCGGGCTATCCG-TGAAAGTT 525  
 ITS-Sv3 CGCCCCCGG---GCGCACCGCGAACGGG-GCCGCGAGGGGCGGGCTATCCG-TGAAAGTT 529  
 ITS-S11 CGCCCCCGG---GCGCACCGCGAACGGG-GCCACGAGGGGCGGGCTATCCG-TGAAAGTT 526  
 ITS-S12 CGCCCCCGG---GCGCACCGCGAACGGG-GCCACGAGGGGCGGGCTATCCG-TGAAAGTT 523  
 ITS-S13 CGCCCCCGG---GCGCACCGCGAACGGG-GCCACGAGGGGCGGGCTATCCG-TGAAAGTT 525  
 ITS-Sb2 CGCCCCCGG---GCGCACCGCGAACGGGTGCCACGAGGGGCGGGCTATCCG-TGAAAGTT 530  
 ITS-Sb3 CGCCCCCGG---GCGCACCGCGAACGGG-GCCACGAGGGGCGGGCTATCCGCTGAAAGTT 525  
 ITS-Sb1 CGCCCCCGG---GCGCACCGCGAACGGG-GCCACGAGGGGCGGGCTATCCG-TGAAAGTT 528  
 ITS-Sb4 CGCCCCCGG---GCGCACCGCGAACGAG-GCCACGAGGGGCGGGCTATCCG-TGAAAGTT 527  
 ITS-St1 CTCCCGCCGAGTGCGCACCGCGAACGGGACCG-ACGGGGCAGGCTATCAG-TGAAAGTT 525  
 ITS-St4 CTCCCGCCGAGTGCGCACCGCGAACGGGACCG-ACGGGGCAGGCTATCAG-TGAAAGTT 525  
 ITS-St2 CTCCCGCCGAGTGCGCACCGCGAACGGGACCG-ACGGGGCAGGCTATCAG-TGAAAGTT 526  
 ITS-St3 CTCCCGCCGAGTGCGCACCGCGAACGGGACCG-ACGGGGCAGGCTATCAG-TGAAAGTT 526  
 \* \* \* \* \* \*\*\*\*\* \* \* \* \*\*\*\*\*

ITS-Sv4 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 586  
 ITS-Sv5 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 585  
 ITS-Sv1 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 584  
 ITS-Sv2 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 584  
 ITS-Sv3 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 588  
 ITS-S11 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 585  
 ITS-S12 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 582  
 ITS-S13 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 584  
 ITS-Sb2 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 589  
 ITS-Sb3 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 584  
 ITS-Sb1 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 587  
 ITS-Sb4 TTCCTTGCGCTTTCCGCGCCGGGGTTCG-TTGTTTGCGCGCGGCCCGGCACCCGAGGGA 586  
 ITS-St1 TTCCTTGCGCTTTCCGCGCCGGGGTTCATTTGTTTGCGCACGGCCTGGCACCCGAGGGT 585  
 ITS-St4 TTCCTTGCGCTTTCCGCGCCGGGGTTCATTTGTTTGCGCACGGCCTGGCACCCGAGGGT 585  
 ITS-St2 TTCCTTGCGCTTTCCGCGCCGGGGTTCATTTGTTTGCGCACGGCCTGGCACCCGAGGGT 586  
 ITS-St3 TTCCTTGCGCTTTCCGCGCCGGGGTTCATTTGTTTGCGCACGGCCTGGCACCCGAGGGT 586  
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ITS-Sv4 TCGGGCCGCTCGCGAGACGGGCGGAGGCGGGGTGCCCCC-TCCCGACGCCCGTGGAGA 645  
 ITS-Sv5 TCGGGCCGCTCGCGAGACGGGCGGAGGCGGGGTGCCCCC-TCCCGACGCCCGTGGAGA 644  
 ITS-Sv1 TCGGGCCGCTCGCGAGACGGTGCAGGCGGGGTGCCCCC-TCCCGACGCCCGTGGAGA 643  
 ITS-Sv2 TCGGGCCGCTCGCGAGACGGGCGGAGGCGGGGTGCCCCC-TCCCGACGCCCGTGGAGA 643  
 ITS-Sv3 TCGGGCCGCTCGCGAGACGGGCGGAGGCGGGGTGCCCCC-TCCCGACGCCCGTGGAGA 647  
 ITS-S11 TCGGGCCGCTCGCGAGACGGGCGGAGGCGGGGTGCCCCC-TCCCGACGCCCGTGGAGA 644  
 ITS-S12 TCGGGCCGCTCGCGAGACGGGCGGAGGCGGGGTGCCCCC-TCCCGACGCCCGTGGAGA 641

ITS-S13 TCGGGCCGCTCGCGAGACGGGGCCGAGGCGGGGTTGCCCCC-TCCCGACGCCCGTGGAGA 643  
 ITS-Sb2 CCGGGCCGCTCGCGAAACGGGGCCGAGACGGGGTGGCCCCCTCCCGACGCCCGGGAGA 649  
 ITS-Sb3 CCGGGCCGCTCGCGAAACGGGGCCGAGACGGGGTGGCCCCCTCCCGACGCCCGGGAGA 644  
 ITS-Sb1 CCGGGCCGCTCGCGAAACGGGGCCGAGACGGGGTGGCCCCCTCCCGACGCCCGGGAGA 647  
 ITS-Sb4 CCGGGCCGCTCGCGAAACGGGGCCGAGACGGGGTGGCCCCCTCCCGACGCCCGGGAGA 646  
 ITS-St1 GCAAGCCGGTCGCGGAACGAGGCCGAGAAAGGGTTGCCCCC-GCCCGCGCCCGTGGGAA 644  
 ITS-St4 GCAAGCCGGTCGCGGAACGAGGCCGAGAAAGGGTTGCCCCC-GCCCGCGCCCGTGGGAA 644  
 ITS-St2 GCAAGCCGGTCGCGGAACGAGGCCGAGAAAGGGTTGCCCCC-GCCCGCGCCCGTGGGAA 645  
 ITS-St3 GCAAGCCGGTCGCGGAACGAGGCCGAGAAAGGGTTGCCCCC-GCCCGCGCCCGTGGGAA 645

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ITS-Sv4 GTAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 705  
 ITS-Sv5 GTAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 704  
 ITS-Sv1 GTAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 703  
 ITS-Sv2 GTAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 703  
 ITS-Sv3 GTAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 707  
 ITS-S11 GAAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAA 704  
 ITS-S12 GAAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 701  
 ITS-S13 GAAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 703  
 ITS-Sb2 GAAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 709  
 ITS-Sb3 GAAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 704  
 ITS-Sb1 GAAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 707  
 ITS-Sb4 GAAACGAGTTCGCGGGTCGTCTGCAGGATTCGACAATGATCCTTCCGCAGGTTACCTAC 706  
 ITS-St1 GAAACGAGTTCGCGGGTCATCTGCAGGATTCGTCAATGATCCTTCCGCAGGTTACCTAC 704  
 ITS-St4 GAAACGAGTTCGCGGGTCATCTGCAGGATTCGTCAATGATCCTTCCGCAGGTTACCTAC 704  
 ITS-St2 GAAACGAGTTCGCGGGTCATCTGCAGGATTCGTCAATGATCCTTCCGCAGGTTACCTAC 705  
 ITS-St3 GAAACGAGTTCGCGGGTCATCTGCAGGATTCGTCAATGATCCTTCCGCAGGTTACCTAC 705

\* \* \* \* \*

ITS-Sv4 GGAAACCTTGTTACGATTTTT-ACTTCCAA 734  
 ITS-Sv5 GGAAACCTTGTTACGATTTTT-ACTTCCAA 733  
 ITS-Sv1 GGAAACCTTGTTACGATTTTT-ACTTCCAA 732  
 ITS-Sv2 GGAAACCTTGTTACGATTTTT-ACTTCCAA 732  
 ITS-Sv3 GGAAACCTTGTTACGATTTTT-ACTTCCAA 736  
 ITS-S11 GGAAACCTTGTTACGATTTTT-ACTTCCAA 733  
 ITS-S12 GGAAACCTTGTTACGATTTTT-ACTTCCAA 730  
 ITS-S13 GGAAACCTTGTTACGATTTTT-ACTTCCAA 732  
 ITS-Sb2 GGAAACCTTGTTACGATTTTT-ACTTCCAA 738  
 ITS-Sb3 GGAAACCTTGTTACGATTTTT-ACTTCCAA 733  
 ITS-Sb1 GGAAACCTTGTTACGATTTTT-ACTTCCAA 736  
 ITS-Sb4 GGAAACCTTGTTACGATTTTTACTTCCAA 736  
 ITS-St1 GGAAACCTTGTTACGATTTTTAACTTCCAA 734  
 ITS-St4 GGAAACCTTGTTACGATTTTTA-CTTCCAA 733  
 ITS-St2 GGAAACCTTGTTAC-ATTTTTACTTCCAA 734  
 ITS-St3 GGAAACCTTGTTACGATTTTTACTTCCAA 735

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### The size of the ITS region

Sequence 1: ITS-St1 734 bp

Sequence 2: ITS-St2 734 bp

Sequence 3: ITS-St3 735 bp

Sequence 4: ITS-St4 733 bp

Sequence 5: ITS-Sl1 733 bp

Sequence 6: ITS-Sl2 730 bp

Sequence 7: ITS-Sl3 732 bp

Sequence 8: ITS-Sv1 732 bp

Sequence 9: ITS-Sv2 732 bp

Sequence 10: ITS-Sv3 736 bp

Sequence 11: ITS-Sv4 734 bp

Sequence 12: ITS-Sv5 733 bp

Sequence 13: ITS-Sb1 736 bp

Sequence 14: ITS-Sb2 738 bp

Sequence 15: ITS-Sb3 733 bp

Sequence 16: ITS-Sb4 736 bp



### The pairwise of similarity score

Sequences (1:2) Aligned. Score: 98	Sequences (4:6) Aligned. Score: 88	Sequences (8:9) Aligned. Score: 99
Sequences (1:3) Aligned. Score: 98	Sequences (4:7) Aligned. Score: 88	Sequences (8:10) Aligned. Score: 99
Sequences (1:4) Aligned. Score: 99	Sequences (4:8) Aligned. Score: 88	Sequences (8:11) Aligned. Score: 98
Sequences (1:5) Aligned. Score: 87	Sequences (4:9) Aligned. Score: 89	Sequences (8:13) Aligned. Score: 95
Sequences (1:6) Aligned. Score: 88	Sequences (4:10) Aligned. Score: 88	Sequences (8:14) Aligned. Score: 95
Sequences (1:7) Aligned. Score: 88	Sequences (4:11) Aligned. Score: 88	Sequences (8:15) Aligned. Score: 95
Sequences (1:8) Aligned. Score: 87	Sequences (4:12) Aligned. Score: 88	Sequences (8:16) Aligned. Score: 95
Sequences (1:9) Aligned. Score: 88	Sequences (4:13) Aligned. Score: 88	Sequences (9:10) Aligned. Score: 99
Sequences (1:10) Aligned. Score: 88	Sequences (4:14) Aligned. Score: 89	Sequences (9:11) Aligned. Score: 99
Sequences (1:11) Aligned. Score: 88	Sequences (4:15) Aligned. Score: 89	Sequences (9:12) Aligned. Score: 99
Sequences (1:12) Aligned. Score: 87	Sequences (4:16) Aligned. Score: 88	Sequences (9:13) Aligned. Score: 95
Sequences (1:13) Aligned. Score: 88	Sequences (5:6) Aligned. Score: 99	Sequences (9:14) Aligned. Score: 95
Sequences (1:14) Aligned. Score: 88	Sequences (5:7) Aligned. Score: 99	Sequences (9:15) Aligned. Score: 95
Sequences (1:15) Aligned. Score: 88	Sequences (5:8) Aligned. Score: 97	Sequences (9:16) Aligned. Score: 95
Sequences (1:16) Aligned. Score: 88	Sequences (5:9) Aligned. Score: 98	Sequences (10:11) Aligned. Score: 99
Sequences (2:3) Aligned. Score: 99	Sequences (5:10) Aligned. Score: 98	Sequences (10:12) Aligned. Score: 99
Sequences (2:4) Aligned. Score: 98	Sequences (5:11) Aligned. Score: 97	Sequences (10:13) Aligned. Score: 95
Sequences (2:5) Aligned. Score: 87	Sequences (5:12) Aligned. Score: 97	Sequences (10:14) Aligned. Score: 95
Sequences (2:6) Aligned. Score: 87	Sequences (5:13) Aligned. Score: 95	Sequences (10:15) Aligned. Score: 95
Sequences (2:7) Aligned. Score: 88	Sequences (5:14) Aligned. Score: 95	Sequences (10:16) Aligned. Score: 95
Sequences (2:8) Aligned. Score: 88	Sequences (5:15) Aligned. Score: 95	Sequences (11:12) Aligned. Score: 99
Sequences (2:9) Aligned. Score: 88	Sequences (5:16) Aligned. Score: 95	Sequences (11:13) Aligned. Score: 95
Sequences (2:10) Aligned. Score: 88	Sequences (6:7) Aligned. Score: 99	Sequences (11:14) Aligned. Score: 95
Sequences (2:11) Aligned. Score: 88	Sequences (6:8) Aligned. Score: 97	Sequences (11:15) Aligned. Score: 95
Sequences (2:12) Aligned. Score: 88	Sequences (6:9) Aligned. Score: 98	Sequences (11:16) Aligned. Score: 95
Sequences (2:13) Aligned. Score: 88	Sequences (6:10) Aligned. Score: 98	Sequences (12:13) Aligned. Score: 95
Sequences (2:14) Aligned. Score: 88	Sequences (6:11) Aligned. Score: 97	Sequences (12:14) Aligned. Score: 95
Sequences (2:15) Aligned. Score: 88	Sequences (6:12) Aligned. Score: 97	Sequences (12:15) Aligned. Score: 95
Sequences (2:16) Aligned. Score: 88	Sequences (6:13) Aligned. Score: 95	Sequences (12:16) Aligned. Score: 95
Sequences (3:4) Aligned. Score: 99	Sequences (6:14) Aligned. Score: 95	Sequences (13:14) Aligned. Score: 99
Sequences (3:5) Aligned. Score: 88	Sequences (6:15) Aligned. Score: 95	Sequences (13:15) Aligned. Score: 99
Sequences (3:6) Aligned. Score: 88	Sequences (6:16) Aligned. Score: 95	Sequences (13:16) Aligned. Score: 98
Sequences (3:7) Aligned. Score: 88	Sequences (7:8) Aligned. Score: 97	Sequences (14:15) Aligned. Score: 99
Sequences (3:8) Aligned. Score: 88	Sequences (7:9) Aligned. Score: 98	Sequences (14:16) Aligned. Score: 98
Sequences (3:9) Aligned. Score: 89	Sequences (7:10) Aligned. Score: 98	Sequences (15:16) Aligned. Score: 99
Sequences (3:10) Aligned. Score: 88	Sequences (7:11) Aligned. Score: 97	
Sequences (3:11) Aligned. Score: 88	Sequences (7:12) Aligned. Score: 97	
Sequences (3:12) Aligned. Score: 88	Sequences (7:13) Aligned. Score: 95	
Sequences (3:13) Aligned. Score: 88	Sequences (7:14) Aligned. Score: 95	
Sequences (3:14) Aligned. Score: 88	Sequences (7:15) Aligned. Score: 95	
Sequences (3:15) Aligned. Score: 88	Sequences (7:16) Aligned. Score: 95	
Sequences (3:16) Aligned. Score: 88	Sequences (8:12) Aligned. Score: 98.	
Sequences (4:5) Aligned. Score: 88		

The *rbcl* sequence of *S. thorelii*, *S. nux-vomica*,  
*S. lucida* and *S. nux-blanda*

>*rbcl-St1*

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>*rbcl-St2*

GCTGGTGTAAAGAGTACAAATTGACTTTATTACTCTCAATACGAAACCAAAGATACTGATATCTTGGCACCATTCCGAGTAACTCCTCAACCCGGAGTT  
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>*rbcl-St3*

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>**rbcl-St4**

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 GAATTAAT

>**rbcl-Sl1**

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**>rbcl-Sl2**

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CTTTAGCAAATCGAGGTAACCTCTTAAAAAACATTGTGTA AAAAACCTCCCTTAATGAAGGGCGTGATCTTGCTGCTGAGGGTAATGATATTATCCGTGAGGC  
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**>rbcl-Sl3**

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CCGCTCATGGCATCCAAGTTGAGAGAGATAAATTGAACAAGTATGGTCGACCTCTGTTGGGATGACTATTAAACCTAAATTGGGGTTATCCGCTAAAACT  
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**>rbcl-Sv1**

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 AA

>*rbcl-Sv2*

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จุฬาลงกรณ์มหาวิทยาลัย

>*rbcl-Sv3*

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**>rbcl-Sv4**

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**>rbcl-Sv5**

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**>rbcl-Sb1**

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>*rbcl-Sb2*

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>*rbcl-Sb3*

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 TAAA



>*rbcl-Sb4*

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The multiple sequence alignment of *rbcl* gene

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rbcl-Sb3      GCTGGTGTAAAGAGGACAA-TTGAC-ATATTATACTCC-GGAATACGAAACCCAAA-GATA 56
rbcl-Sb4      GCTGGTGTAAAGAGGACAA-TTGAC-TTATTATACTCC-GGAATACGAAACCCAAA-GATA 56
rbcl-Sv1      GCTGGTGTAAAGAGTACAA-TTGAC-TTATTATACTCC-TGAATACGAAACCCAAA-GATA 56
rbcl-Sv5      GCTGGTGTAAAAGGACAAATTGAC-TTATTATACTCC-TGAATACGAAACCCAAA-GATA 57
rbcl-Sv4      GCTGGTGT-TAAGAGGACAAATTGACATTATTATACTCC-GGAATACGAAACCCAAA-AATA 57
rbcl-Sv2      GCTGGTGTAAAGAGTACAC-TTGAC-TTATTATACTCCTGGAATACGAAACCCAAA-GTTA 57
rbcl-Sv3      GCTGGTGTAAAGAGTACAA-TTGAC-TTATTATACTCCTGGAATACGAAACCCAAA-GATA 57
rbcl-Sb1      GCTGGTGTAAAAGAGTACAAATTGAC-ATTATATACTCC-TGAATACGAAACCCAAA-GATA 57
rbcl-Sb2      GCTGGTGTAAAAGAGTACAAATTGAC-ATATTATACTCC-TGAATACGAAACCCAAA-GATA 57
rbcl-Sl2      GCTGGTGTAAAGAGTACAAATTGAC-TTATTATACTCC-TGAATACGAAACCCAAA-GATA 57
rbcl-Sl3      GCTGGTGTAAAGAGTACAAATTGAC-TTATTATACTCC-TGAATACGAAACCCAAA-GATA 57
rbcl-Sl1      GCTGGTGTAAAGAGTACAAATTGAC-TTATTATACTCC-TGAATACGAAACCCAAA-GATA 57
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rbcl-St4      GCTGGTGTAAAGAGCACAA-TTGAC-TTATTATACTCC-TCAATACGAAACCCAAA-GATA 56
rbcl-St2      GCTGGTGTAAAGAGTACAAATTGACTTTATTATACTCC-TCAATACGAAACCCAAA-GATA 58
rbcl-St3      GCTGGTGTAAAGAGTACAAATTGACTTTATTATACTCC-TCAATACGAAACCCAAAAGATA 59
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rbcl-Sb3      -CTGATATCTTGG-CACCATTCCG-AGTAAC-TCCTCAACCCGGAAATCCACCTGAAGA 112
rbcl-Sb4      -CTGATATCTTGG-CAGCATTCCG-AGTAAC-TCCTCAACCCGGGAGTTCACCTGAAGA 112
rbcl-Sv1      -CTGATATCTTGG-CAGCATTCCGGAGTAAC-TCCTCAACCCGGAG-TTCCACCTGAAAA 112
rbcl-Sv5      -CTGATATCTTGG-CACCATTCCG-AGTAAC-TCCTCAACCCGGAGATTCACCTGAAGA 113
rbcl-Sv4      TCTGATATCTTGG-CAACATTCCG-AGTAAC-TCCTCAACCCGGAGTTCACCTGAAGA 114
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rbcl-Sb1      -CTGATATCTTGG-CAGCATTCCG-AGTAAC-TCCTCAACCCGGAGTTCACCTGAAGA 113
rbcl-Sb2      -CTGATATCTTGG-CAGCATTCCG-AGTAAC-TCCTCAACCCGGAGTTCACCTGAAAA 113
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rbcl-St4      -CTGATATCTTGG-CAGCATTCCG-AGTAAC-TCCTCAACCCGGAG-TTCCACCTGAAGA 111
rbcl-St2      -CTGATATCTTGG-CACCATTCCG-AGTAACCTCCTCAACCCGGAG-TTCCACCTGAAAA 114
rbcl-St3      -CTGATATCTTGGGCAGCATTCCG-AGTAAC-TCCTCAACCCGGAGATTCACCTGAAGA 116
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rbcl-Sb3      AACAGGGGCCCG-AGTAGTGCCG-AATCTTCTACTGGGACATGGA-CAATGTGTGG-A 168
rbcl-Sb4      AGCAAGGGCCCC-AGTAGTGCCG-AATCTTCTACTGGTACATGGA-CAACTGTGTGG-A 168
rbcl-Sv1      ACCAGGGGCCCG-AGTAGTGCCG-AATCTTCTACTGGGACATGGA-AACTGTGTGG-A 168
rbcl-Sv5      AGCAGGGGCCCG-AGTAGTGCCGGAATCTTCTACTGGTACATGGA-CAACTGTGTGG-A 170
rbcl-Sv4      AGCAGGGGCCCG-AGTAGTGCCG-AATCTTCTACTGGTACATGGA-CAACTGTGTGG-A 170
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rbcl-St4      ACCAGGGGCCCGAGTAGTGCCG-AATCTTCTACTGGTACATGGA-AACTGTGTGG-A 168
rbcl-St2      AGCAGGGGCCCG-AGTAGTGCCG-AATCTTCTACTGGTACATGGA-CAACTGTGTGG-A 170
rbcl-St3      AGCAGGGGCCCG-AGTAGTGCCG-AATCTTCTACTGGTACATGGA-CAACTGTGTGG-A 172
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rbcl-Sb3      CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGTACA-ATA-TCGACCC 223
rbcl-Sb4      CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGTACC-ATA-TCGAGCC 223
rbcl-Sv1      CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGTACA-ATA-TCGAGCC 223
rbcl-Sv5      CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGTACC-ATA-TCGAGCC 225
rbcl-Sv4      CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGTACC-ATA-TCGAGCC 225
rbcl-Sv2      CCGA-TGGACTTACCAGCC-TTGATCGTTACAAAAGGGCGCTGTACCCATAATCGAGCC 228
rbcl-Sv3      CCGA-TGGACTTACCAGCC-TTGATCGTTACAAAAGGGCGCTGTACC-ATAATCGAGCC 227
rbcl-Sb1      CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGTACA-ATA-TCGAGCC 224

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rbcL-Sb2          CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGCCGCTGCTACC-ATA-TCGAGCC 224
rbcL-S12          CCGA-TGGACTTACCAGCCCTTGATCGTTACAAA-GGGCGCTGCTACC-ATA-TCGAGCC 227
rbcL-S13          CCGA-TGGACTTACCAGCCCTTGATCGTTACAAA-GGGCGCTGCTACC-ATA-TCGAGCC 227
rbcL-S11          CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGCTACA-ATA-TCGAGCC 225
rbcL-St1          CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGCTAGA-TAT-TCCAGCC 226
rbcL-St4          CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGCCGCTGCTACA-ATA-TCGAGCC 223
rbcL-St2          CCGA-TGGACTTACCAGCC-TTGATCGTTACAAA-GGGCGCTGCTACC-ATA-TCGAGCC 225
rbcL-St3          CCGAATGGACTTACCAGCCCTTGATCGTTACAAA-GGGCGCTGCTACC-ATA-TCGAGCC 229
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rbcL-Sb3          TGTGCTGGAGAAGAAGATCAATTTA-TTGCTTAAGTAGCTTACCCCTTAGACCTTTTTG 282
rbcL-Sb4          TGTGCTGGAGAAGAAGATCAATTTA-TTGCTTAAGTAGCTTACCCCTTAGACCTTTTTG 282
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rbcL-Sv5          TGTGCTGGAGAAGAAGATCAATATA-TTGCTTAAGTAGCTTACCCCTTAGACCTTTTTG 284
rbcL-Sv4          TGTGCTGGAGAAGAAGATCAATATA-TTGCTTAAGTAGCTTACCCCTTAGACCTTTTTG 284
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rbcL-Sv3          TGTGCTGGAGAAGAAGATCAATATAATTGCTTAAGTAGCTTACCCCTTAGACCTTTTTG 287
rbcL-Sb1          TGTGCTGGAGAAGAAGATCAATTTA-TTGCTTATGTAGCTTACCCCTTAGACCTTTTTG 283
rbcL-Sb2          TGTGCTGGAGAAGAAGATCAATTTA-TTGCTTAAGTAGCTTACCCCTTAGACCTTTTTG 283
rbcL-S12          TGTGCTGGAGAAGAAGATCAATATA-TTGCTTAAGTAGCTTACCCCTTAGACCTTTTTG 286
rbcL-S13          TGTGCTGGAGAAGGAGATCAATATA-TTGCTTAAGTAGCTTACCCCTTAGACCTTTTTG 286
rbcL-S11          TGTGCTGGAGAAGAAGACCAATAAAA-TTGCTTAAGTAGCTTATCCCTTAGACCTTTTTG 284
rbcL-St1          TGTGCTGGAGAAGAAGACCAATAAAA-TTGCTTAAGTAGCTTATCCCTTAGACCTTTTTG 285
rbcL-St4          TGTGCTGGAGAAGAAGATCAATATA-TTGCTTATGTAGCTTATCCCTTAGACCTTTTTG 282
rbcL-St2          TGTGCTGGAGAAGAAGATCAATATA-TTGCTTATGTAGCTTATCCCTTAGACCTTTTTG 284
rbcL-St3          TGTGCTGGAGAAGAAGATCAATATA-TTGCTTATGTAGCTTATCCCTTAGACCTTTTTG 288
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rbcL-Sb4          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 342
rbcL-Sv1          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 342
rbcL-Sv5          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 344
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rbcL-Sv3          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 347
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rbcL-S11          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 344
rbcL-St1          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 345
rbcL-St4          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 342
rbcL-St2          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 344
rbcL-St3          AAGAAGGTTCTGTTACTAACATGTTTACTTCCATTGTAGGTAATGTATTTGGGTTCAAAG 348
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rbcL-Sb3          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 402
rbcL-Sb4          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 402
rbcL-Sv1          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 402
rbcL-Sv5          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 404
rbcL-Sv4          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 404
rbcL-Sv2          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 408
rbcL-Sv3          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 407
rbcL-Sb1          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 403
rbcL-Sb2          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 403
rbcL-S12          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 406
rbcL-S13          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 406
rbcL-S11          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 404
rbcL-St1          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 405
rbcL-St4          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 402
rbcL-St2          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 404
rbcL-St3          CCCTACGCGCTCTACGCTGGAAGATTTGCGAATCCCGTTGCTTATATTTAAAACCTTCC 408
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*rbcL*-Sb3 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 462  
*rbcL*-Sb4 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 462  
*rbcL*-Sv1 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 462  
*rbcL*-Sv5 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 464  
*rbcL*-Sv4 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 464  
*rbcL*-Sv2 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 468  
*rbcL*-Sv3 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 467  
*rbcL*-Sb1 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 463  
*rbcL*-Sb2 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 463  
*rbcL*-S12 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 466  
*rbcL*-S13 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 466  
*rbcL*-S11 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 464  
*rbcL*-St1 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 465  
*rbcL*-St4 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 462  
*rbcL*-St2 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 464  
*rbcL*-St3 AAGGCCCGCCTCATGGCATCCAAGTTGAGAGAGATAAAATGAACAAGTATGGTCGACCTC 468  
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*rbcL*-Sb3 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 522  
*rbcL*-Sb4 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 522  
*rbcL*-Sv1 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 522  
*rbcL*-Sv5 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 524  
*rbcL*-Sv4 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 524  
*rbcL*-Sv2 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 528  
*rbcL*-Sv3 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 527  
*rbcL*-Sb1 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 523  
*rbcL*-Sb2 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 523  
*rbcL*-S12 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 526  
*rbcL*-S13 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 526  
*rbcL*-S11 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 524  
*rbcL*-St1 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 525  
*rbcL*-St4 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 522  
*rbcL*-St2 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 524  
*rbcL*-St3 TGTGGGATGTACTATTTAAACCTAAATGGGGTTATCCGCTAAAAACTACGGTAGGGCAG 528  
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*rbcL*-Sb3 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 581  
*rbcL*-Sb4 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 581  
*rbcL*-Sv1 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 581  
*rbcL*-Sv5 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 583  
*rbcL*-Sv4 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 583  
*rbcL*-Sv2 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 587  
*rbcL*-Sv3 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 586  
*rbcL*-Sb1 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 582  
*rbcL*-Sb2 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 582  
*rbcL*-S12 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 586  
*rbcL*-S13 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 585  
*rbcL*-S11 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 583  
*rbcL*-St1 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 584  
*rbcL*-St4 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 581  
*rbcL*-St2 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 583  
*rbcL*-St3 TTTATGAATGCTTCGTGGTGGACTTGA-TTTTACCAAAGATGATGAAAACGTGAACTCC 587  
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*rbcL*-Sb3 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 641  
*rbcL*-Sb4 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 641  
*rbcL*-Sv1 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 641  
*rbcL*-Sv5 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 643  
*rbcL*-Sv4 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 643  
*rbcL*-Sv2 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 647  
*rbcL*-Sv3 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 646  
*rbcL*-Sb1 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 642  
*rbcL*-Sb2 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCCTTTATAAAGCA 642

*rbcL*-S12 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTATAAAGCA 646  
*rbcL*-S13 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTATAAAGCA 645  
*rbcL*-S11 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTATAAAGCA 643  
*rbcL*-St1 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTATAAAGCA 644  
*rbcL*-St4 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTATAAAGCA 641  
*rbcL*-St2 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTATAAAGCA 643  
*rbcL*-St3 CAACCATTTATGCGTTGGAGAGATCGTTTCTTATTTTGTGCCGAAGCACTTTATAAAGCA 647  
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*rbcL*-Sb3 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 701  
*rbcL*-Sb4 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 701  
*rbcL*-Sv1 CAGACTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 701  
*rbcL*-Sv5 CAGACTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 703  
*rbcL*-Sv4 CAGACTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 703  
*rbcL*-Sv2 CAGACTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 707  
*rbcL*-Sv3 CAGACTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 706  
*rbcL*-Sb1 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 702  
*rbcL*-Sb2 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 702  
*rbcL*-S12 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 706  
*rbcL*-S13 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 705  
*rbcL*-S11 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 703  
*rbcL*-St1 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 704  
*rbcL*-St4 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 701  
*rbcL*-St2 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 703  
*rbcL*-St3 CAGGCTGAAACCGGTGAAATCAAAGGACATTACTTGAATGCTACTGCAGGTACATGCGAA 707  
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*rbcL*-Sb3 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 761  
*rbcL*-Sb4 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 761  
*rbcL*-Sv1 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 761  
*rbcL*-Sv5 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 763  
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*rbcL*-Sv3 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 766  
*rbcL*-Sb1 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 762  
*rbcL*-Sb2 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 762  
*rbcL*-S12 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 766  
*rbcL*-S13 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 765  
*rbcL*-S11 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 763  
*rbcL*-St1 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 764  
*rbcL*-St4 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 761  
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*rbcL*-St3 GAAATGATCAAAGAGCTGTATTTGCTAGAGAATTGGGAGTTCCTATCGTAATGCATGAC 767  
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*rbcL*-Sb3 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 821  
*rbcL*-Sb4 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 821  
*rbcL*-Sv1 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 821  
*rbcL*-Sv5 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 823  
*rbcL*-Sv4 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 823  
*rbcL*-Sv2 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 827  
*rbcL*-Sv3 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 826  
*rbcL*-Sb1 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 822  
*rbcL*-Sb2 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 822  
*rbcL*-S12 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 826  
*rbcL*-S13 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 825  
*rbcL*-S11 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 823  
*rbcL*-St1 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 824  
*rbcL*-St4 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 821  
*rbcL*-St2 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 823  
*rbcL*-St3 TACTTAACAGGGGGATTCACTGCAAATACTAGCTTAGCTCATTATTGCCGAGATAATGGC 827  
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*rbcL*-Sb3 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 881  
*rbcL*-Sb4 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 881  
*rbcL*-Sv1 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 881  
*rbcL*-Sv5 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 883  
*rbcL*-Sv4 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 883  
*rbcL*-Sv2 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 887  
*rbcL*-Sv3 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 886  
*rbcL*-Sb1 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 882  
*rbcL*-Sb2 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 882  
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*rbcL*-Sl1 CTACTTCTTCACATTCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 883  
*rbcL*-St1 CTACTTCTTCACATCCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 884  
*rbcL*-St4 CTACTTCTTCACATCCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 881  
*rbcL*-St2 CTACTTCTTCACATCCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 883  
*rbcL*-St3 CTACTTCTTCACATCCACCGTGAATGCATGCAGTTATTGATAGACAGAAGAATCATGGT 887  
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*rbcL*-Sb3 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 941  
*rbcL*-Sb4 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 941  
*rbcL*-Sv1 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 941  
*rbcL*-Sv5 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 943  
*rbcL*-Sv4 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 943  
*rbcL*-Sv2 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 947  
*rbcL*-Sv3 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 946  
*rbcL*-Sb1 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 942  
*rbcL*-Sb2 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 942  
*rbcL*-Sl2 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 946  
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*rbcL*-Sl1 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 943  
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*rbcL*-St4 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 941  
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*rbcL*-St3 ATGCACTTCCGCGTACTAGCTAAAGCGTTACGTATGTCTGGTGGAGATCATATTCACGCT 947  
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*rbcL*-Sb3 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGGGCTTTGTTGATTTA 1001  
*rbcL*-Sb4 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGGGCTTTGTTGATTTA 1001  
*rbcL*-Sv1 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGGGCTTTGTTGATTTA 1001  
*rbcL*-Sv5 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGGGCTTTGTTGATTTA 1003  
*rbcL*-Sv4 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1003  
*rbcL*-Sv2 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1007  
*rbcL*-Sv3 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1006  
*rbcL*-Sb1 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1002  
*rbcL*-Sb2 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1002  
*rbcL*-Sl2 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1006  
*rbcL*-Sl3 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1005  
*rbcL*-Sl1 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGGGCTTTGTTGATTTA 1003  
*rbcL*-St1 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1004  
*rbcL*-St4 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1001  
*rbcL*-St2 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAAACATCACTTTGGGCTTTGTTGATTTA 1003  
*rbcL*-St3 GGTACTGTAGTAGGTAACCTTGAAGGCGAAAGAGACATCACTTTGGGCTTTGTTGATTTA 1007  
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*rbcL*-Sb3 CTGGCGGGATGATTTTATTGAAAAA-GATCGAAGTCCC-GGTATTTATTT-CACTCAAAG 1058  
*rbcL*-Sb4 CTGGCGGGATGATTTTATTGAAAAA-GATCGAAGTCCC-GGTATTTATTT-CACTCAA-G 1057  
*rbcL*-Sv1 CTGCGTG-ATGATTTTATTGAAAAA-GATCGAAGTCCC-GGTATTTATTT-CACTCAA-G 1056  
*rbcL*-Sv5 CTGCGGGGATGATTTTATTGAAAAA-GATCGAAGTCCC-GGTATTTATTT-CACTCAA-G 1059  
*rbcL*-Sv4 CTGGCGGGATGATTTTATTGAAAAAAGATCGAAGTCCC-CGGTATTTATTTTCACTCAA-G 1062  
*rbcL*-Sv2 CTG-CGGGATGATTTTATTGAAAAAATCGAAGTCCC-GGTATTTATTT-CACTCAA-G 1063  
*rbcL*-Sv3 CTG-CGGGATGATTTTATTGAAAAAATCGAAGTCCC-GGTATTTATTT-CACTCAA-G 1062  
*rbcL*-Sb1 CTGCGGGGATGATTTTATTGAAAAA-GATCGAAGTCCC-GGTATTTATTT-CACTCAA-G 1058  
*rbcL*-Sb2 CTGCGTG-ATGATTTTATTGAAAAA-GATCGAAGTCCC-GGTATTTATTT-CACTCAA-G 1057

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rbcL-S12      CTGCCGGGATGATTTTATTGAAAAA-GATCGAAGTCCC-CGTATTTATTT-CACTCAA-G 1062
rbcL-S13      CTGCCGGGATGATTTTATTGAAAAA-GATCGAAGTCCC-CGTATTTATTT-CACTCAA-G 1061
rbcL-S11      CTGCCGTGATGATTTTATTGAAAAA-GATCGAAGTCCC-CGTATTTATTT-CACTCAA-A 1059
rbcL-St1      CTGGCGGGAGGATTTTATTGAAAAA-GATCCAAACCCCGGTTGTTTATTT-CACTCAA-G 1061
rbcL-St4      CTG-CGGGATGATTTTATTGAAAAA-GATCCAAACCCCGGTTGTTTATTT-CACTCAA-G 1057
rbcL-St2      CTGGCGTGATGATTTTATTGAAAAA-GATCGAAGCCCCCGGTTGTTTATTT-CACTCAA-A 1060
rbcL-St3      CTGCCGGGATGATTTTATTGAAAAA-GATCGAAACCCCGGTTGTTTATTT-CACTCAA-G 1064
***          * ***** ** * ** * ** ***** *****

rbcL-Sb3      ATTGGG-TCTCTCTACCGGGGTGTATACCTGTGGGCTTCGGGGGGTATTCCACGTTTT 1117
rbcL-Sb4      ATTGGG-TCTCTCTACCGGGGTGTATACCTGGGGGCTTCAGGGGGTATTTC-ACCGTTT 1115
rbcL-Sv1      ATTGGG-TCTCTCTACCGGGGTGTATACCTGTGGGCTTCAGGGGGTATTTC-ACG-TTT 1113
rbcL-Sv5      ATTGGGGTCTCTCTACCGGGGTGTATACCTGTGGGCTTCAGGGGGTATTCCACG-TTT 1118
rbcL-Sv4      ATTGGGGTCTCTCTACCGGGGTGTATACCTGTGGGCTTCAGGGGGTATTTC-ACG-TTT 1120
rbcL-Sv2      ATTTGG-TCTCTCTACCGGGGTGTATACCTGTAGGCTTCAGGGGGTATTCCACG-TTT 1121
rbcL-Sv3      ATTGGG-TCTCTCTACCGGGGTGTATACCTGTGGGCTTCAGGGGGTATTCCACG-TTT 1120
rbcL-Sb1      ATTGGG-TCTCTCTACCGGGGTGTATACCTGGTGGCTTCAGGGGGTATTTC-ACG-TTT 1115
rbcL-Sb2      ATTGGG-TCTCTCTACCGGG-TGTATACCTG-TGGCTTCAGGGGG-TATTTC-ACG-TTT 1111
rbcL-S12      ATTGGGGTCTCTCTACCGGGGGTTATACCTGGTGGCTTCAGGGGGTATTTC-ACCGTTT 1121
rbcL-S13      ATTGGGGTCTCTCTACCGGGGGTTATACCTGGTGGCTTCAGGGGGTATTTC-ACCGTTT 1120
rbcL-S11      GATGGGGTCTCTCTACCGGGGGTTATACCTGGTGGCTTCAGGGGGTATTTC-ACCGTTT 1118
rbcL-St1      ATTGGGGTCTCTCTACCGGGGGTTATACCTGGTGGCTTCAGGGGGTATTTC-ACG-TTT 1119
rbcL-St4      ATTGGGGTCTCTCTACCGGGGGTTATACCTGTGGCTTCAGGGGGTATTTC-ACG-TTT 1115
rbcL-St2      AATTGGGTCTCTCTACCGGGGTGTATACCTGTGGGCTTCAGGGGGTATTTC-ACG-TTT 1118
rbcL-St3      A-TGGGTCTCTCTACCGGGG-GTTATACCTGTGG-C TTCAGGGGG-TATTTC-ACG-TTT 1118
* * * ***** ***** * **** ***** ***** * * *

rbcL-Sb3      GGGCA-TATGCCTGGC-TCTGACCAAAAATTTTGGGGAATAAAGCCTTGACTTACAGG 1175
rbcL-Sb4      GGGCA-TATGCCTGGC-TCTGACCAAAAATTTTGGGGGATAGAGGCTGGACCTAACAG- 1172
rbcL-Sv1      GGGCA-TATGCCTGGC-TCTGACCAAGATTTTGGGGGATAGAGGCTGGACCTACA-G 1169
rbcL-Sv5      GGGCA-TATGCCTGGCTCTGACCAAGATTTTGGGGGATAGATGCCTGGACCTACAAG 1177
rbcL-Sv4      GGGCA-TATGCCTGGCTCTGACCAAAAATTTTGGGGGATAGATGCTT--ACTTACA-G 1176
rbcL-Sv2      TGGCAATATGCCTGGC-TCTGACCAAAAATTTTGGGGGATGAATGCTTGACTTACA-G 1179
rbcL-Sv3      TGGCAATATGCCTGGC-TCTGACCAAAAATTTTGGGGGATGAATGCTTGACTTACA-G 1178
rbcL-Sb1      GGCCA-TATGCCTGGCTCTGACCCAGAATTTTGGGGGATGAAGGCTTGACTTACAGG 1174
rbcL-Sb2      GGC-A-TATGCCTGCTCT-GACCAAGATTTTGGGGGATGAATCCTTGACTTACAG- 1167
rbcL-S12      GGCCA-TATGCCTTGC-TCTGACCAAAAATTTTGGGGGATGAAGCC-TGAAC TAACA-G 1177
rbcL-S13      GGCCA-TATGCCTTGC-TCTGACCAAAAATTTTGGGGGATGAAGCC-TGAAC TAACA-G 1176
rbcL-S11      GGCCA-TATGCCTGGC-TCTGACCAAAAATTTTGGGGGATAGAGCC-TGTACTTACA-G 1174
rbcL-St1      GGGCA-TATGCCTGCT-CCTGACCCAGAATTTTGGGGGATAGAGCTGTAACCTACA-G 1176
rbcL-St4      GGGCA-TATGCCTGGC-TCTGACCCAGAATTTTGGGGGATGAAGCTTGACTTACACA-G 1172
rbcL-St2      GGGCA-TATGCCTGGC-TCTGACCAAAAATTTTGGGGGATGAAGCCTGAA-C-TACA-G 1173
rbcL-St3      GG-CA-TATGCCTGGC-TCTGACCAAAA-TTTTGGGGGATGAAGCCTGTA-CCTACA-G 1172
* * ***** ***** ***** * * * * * * * * * * * *

rbcL-Sb3      TTCCGGTGGAGGAAACTTTTAGGACACCCTTGGGGTAATGC-GCC----AGGTGCCG 1229
rbcL-Sb4      TTCCGGGGGAGGAA--CTTTATGGACACCCTTGGGGTAATGC-GCC----AGGTGCCG 1224
rbcL-Sv1      TTCCGGTGGGAGGAA-CTTTTAGGA-CACCCTTGGGGGAAAA-GGCCCAAGGGGCCCT 1226
rbcL-Sv5      TTCCGGTGGGAGGAA-CTTTTAGGAACACCCTTGGGGGAAAA-GGCCCAAGGGGCCCT 1235
rbcL-Sv4      TTCCGGTGGGAGGAA-CTTTAAGGACACCCTTGGGGGAAAA-GGCCCAAGGGGCCCT 1234
rbcL-Sv2      TTCCGGTGGGAGGAA-CTTTTAGGAACACCCTTGGGGGAAAA-GCCTCAGGTGCCCGT 1237
rbcL-Sv3      TTCCGGTGGGAGGAA-CTTTTAGGAACACCCTTGGGGGAAAA-GCCTCAGGTGCCCGT 1236
rbcL-Sb1      TTCCGGTGGGAGGAAACTTTTAGGAACACCCTTGGGGGAAAA-GCGCCA-GGGTGCCC 1232
rbcL-Sb2      TTCCGG--GGGAGAACCTTAAGGA-CACCCTTGGGGGAAAA-GCGCCA-AGGGGCC 1222
rbcL-S12      TTCCGGTGGAGG-AACTTTAAGGAACACCCTTGGGGGAAAA-GGCCCAAGTGCC 1235
rbcL-S13      TTCCGGTGGAGG-AACTTTAAGGAACACCCTTGGGGGAAAA-GGCCCAAGTGCC 1234
rbcL-S11      TTCCGGTGGGAGGAACTTTAAGGACACCCTTGGGGGAAAA-GGCCCAAGGGGCC 1233
rbcL-St1      TTCCGGGGGAGAGAACTTTTAGGACACCCTTGGGGGAAAA-GCCTCAGGTGCCCGT 1236
rbcL-St4      TTC--GGGGAAGGAAACTTTTAGGACACCCCTTGGGGGAAAAAG--GCCCAAGGGGCCG 1227
rbcL-St2      TTCC-GGTGGAAGGAACTTTAAGGACACCCCTTGGGGGTAAT-GCGCCAAGGTGCCCT 1231
rbcL-St3      TTCC-GGTGAGGAACTTTAAGGACACCCCTTGGGGG-AAAT-GGCCCA-GGTGCC 1228
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rbcL-Sb3      TAGC--GAATC-GAGTAG--CTC-TAGAAGCAT-GTGTAAAAG----CTCGTAATGAAGG 1278
rbcL-Sb4      TAGC--GAATC-GAGTAG--CTC-TAGAAGCAT-GTGTAAAAG----CTCGTAATGAAGG 1273
rbcL-Sv1      TAAGC-GAATC-GAGTAG--CTC-TAGAAGCAT-GTGTAAAAG----CTCGTAATGAAGG 1276
rbcL-Sv5      TAACC--AATC-GAGTAG--CTC-TAGAAGCAT-GTGTAAAAG----CTCGTAATGAAGG 1284
rbcL-Sv4      TAACC-AAATC-CAGGAAAATC-TAGAAGCAT-GTGTAAAAG----CTCGTAATGAAGG 1286
rbcL-Sv2      TACCG-AAATC-AGAGAAGCGTC-TAGAAGCAT-GTGTAAAAG----CTCGTAATGAAGG 1289
rbcL-Sv3      TACCG-AAATC-AGAGAAGCGTC-TAGAAGCAT-GTGTAAAAG----CTCGTAATGAAGG 1288
rbcL-Sb1      TAACCCGAATCCGAGTAGCCCTCATAAAACCAT-GTGTAAAAG----CTCGTAATGAAGG 1287
rbcL-Sb2      TAACCAAACC--AGGAGCTCT---AGAAGCAT-GTGTAAAAG----CTCGTAATGAAGG 1272
rbcL-S12      TTTAGCAAATCGAGGTAACCTCTTAAAAAACATTGTGTAAAAAACCTCCCTTAATGAAGG 1295
rbcL-S13      TTTAGCAAATCGAGGTAACCTCTTAAAAAACATTGTGTAAAAAACCTCCCTTAATGAAGG 1294
rbcL-S11      TTAACCAAATCCAGGTAACCTCTTAAAAAACATTGTGTAAAAAACCTCCCTTAATGAAGG 1293
rbcL-St1      TAGCGAAAAACAAGGTATCTCTAAAAAACATTGTGTAAAAAACCTCCCTTAATGAAGG 1295
rbcL-St4      TACCCAA--TCCAGGAATCTCCTGAAAA--CCAT-GTGTAAAAAGCTCGTA--ATGAAGG 1281
rbcL-St2      TAGCCGAAACCAGGATCTCTAAAAAACCATTTGTGTAAAAAACCTCCCTAAATGAAGG 1291
rbcL-St3      TTACCCAATCCAGG-TATCTCTTAAGAA--CCAT-GTGTAAAAAACCTCTGTAATGAAGG 1285
      *           *           *           *           * * * * *           ****

rbcL-Sb3      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1333
rbcL-Sb4      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1328
rbcL-Sv1      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1331
rbcL-Sv5      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1339
rbcL-Sv4      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1341
rbcL-Sv2      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1344
rbcL-Sv3      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1343
rbcL-Sb1      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1342
rbcL-Sb2      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1327
rbcL-S12      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1350
rbcL-S13      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1349
rbcL-S11      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1348
rbcL-St1      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1350
rbcL-St4      GC-GTGATCTT---GCTGCT-GAGGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1336
rbcL-St2      GCTGTGAACTTTGGCCCCCTAGAGGGGAAAGATATTATCCGGGAGGCTAGCAAATGGAGT 1351
rbcL-St3      GC-GTGGATCTTTTGCCGCTAAAAGGTAATGATATTATCCGTGAGGCTAGCAAATGGAGT 1344
      ** ***      *           * * * * * *****

rbcL-Sb3      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTTCAAGCAGTG 1393
rbcL-Sb4      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTTCAAGCAGTG 1388
rbcL-Sv1      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGGTTAATTTTCAAGCAGTG 1391
rbcL-Sv5      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGGTTAATTTTCAAGCAGTG 1399
rbcL-Sv4      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGGTTAATTTTCAAGCAGTG 1401
rbcL-Sv2      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGGTTAATTTTCAAGCAGTG 1404
rbcL-Sv3      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGGTTAATTTTCAAGCAGTG 1403
rbcL-Sb1      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTTCAAGCAGTG 1402
rbcL-Sb2      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTTCAAGCAGTG 1387
rbcL-S12      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTTCAAGCAGTG 1410
rbcL-S13      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGGAGGAGATCAGATTTAATTTTCAAGCAGTG 1409
rbcL-S11      CCTGAACCTTGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTTCAAGCAGTG 1408
rbcL-St1      CCTGAACCTCGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTACCAAGCAGTG 1410
rbcL-St4      CCTGAACCTCGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTACCAAGCAGTG 1396
rbcL-St2      CCTGAACCTCGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTACCAAGCAGTG 1411
rbcL-St3      CCTGAACCTCGCTGCTGCTTGTGAGGTATGGAAGGAGATCAGATTTAATTTACCAAGCAGTG 1404
      ***** *****

rbcL-Sb3      GATACTTTGGATCCGCTTAAGGCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1446
rbcL-Sb4      GATACTTTGGATCCGCTTAAGGCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1441
rbcL-Sv1      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1444
rbcL-Sv5      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1452
rbcL-Sv4      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1454
rbcL-Sv2      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1457
rbcL-Sv3      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1456
rbcL-Sb1      GATACTTTGGATCCGCTTAAGGCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1455
rbcL-Sb2      GATACTTTGGATCCGCTTAAGGCTTAATTACCTTTGTCTCGTAGTGAATTTAAA 1440

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rbcl-Sl2      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTTAATTAAT 1463
rbcl-Sl3      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTTAATTAAT 1462
rbcl-Sl1      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTGAATTAATA 1461
rbcl-St1      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTGAATTAATA 1463
rbcl-St4      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGTGAATTAATA 1449
rbcl-St2      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGT-AATGAAA 1463
rbcl-St3      GATACTTTGGATCCGCTTAAGTCTTAATTACCTTTGTCTCGTAGT-AATGAAA 1456
*****

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### The size of the *rbcl* gene

Sequence 1: *rbcl*-St1 1463 bp  
 Sequence 2: *rbcl*-St2 1463 bp  
 Sequence 3: *rbcl*-St3 1456 bp  
 Sequence 4: *rbcl*-St4 1449 bp  
 Sequence 5: *rbcl*-Sl1 1461 bp  
 Sequence 6: *rbcl*-Sl2 1463 bp  
 Sequence 7: *rbcl*-Sl3 1462 bp  
 Sequence 8: *rbcl*-Sv1 1444 bp  
 Sequence 9: *rbcl*-Sv2 1457 bp  
 Sequence 10: *rbcl*-Sv3 1456 bp  
 Sequence 11: *rbcl*-Sv4 1454 bp  
 Sequence 12: *rbcl*-Sv5 1452 bp  
 Sequence 13: *rbcl*-Sb1 1455 bp  
 Sequence 14: *rbcl*-Sb2 1440 bp  
 Sequence 15: *rbcl*-Sb3 1446 bp  
 Sequence 16: *rbcl*-Sb4 1441 bp



## The pairwise of similarity score

Sequences (1:2) Aligned. Score: 95	Sequences (4:6) Aligned. Score: 96	Sequences (8:11) Aligned. Score: 97
Sequences (1:3) Aligned. Score: 95	Sequences (4:7) Aligned. Score: 95	Sequences (8:12) Aligned. Score: 98
Sequences (1:4) Aligned. Score: 97	Sequences (4:8) Aligned. Score: 96	Sequences (8:13) Aligned. Score: 97
Sequences (1:5) Aligned. Score: 95	Sequences (4:9) Aligned. Score: 95	Sequences (8:14) Aligned. Score: 97
Sequences (1:6) Aligned. Score: 94	Sequences (4:10) Aligned. Score: 95	Sequences (8:15) Aligned. Score: 97
Sequences (1:7) Aligned. Score: 94	Sequences (4:11) Aligned. Score: 95	Sequences (8:16) Aligned. Score: 97
Sequences (1:8) Aligned. Score: 95	Sequences (4:12) Aligned. Score: 95	Sequences (9:10) Aligned. Score: 99
Sequences (1:9) Aligned. Score: 94	Sequences (4:13) Aligned. Score: 96	Sequences (9:11) Aligned. Score: 97
Sequences (1:10) Aligned. Score: 94	Sequences (4:14) Aligned. Score: 96	Sequences (9:12) Aligned. Score: 97
Sequences (1:11) Aligned. Score: 95	Sequences (4:15) Aligned. Score: 95	Sequences (9:13) Aligned. Score: 96
Sequences (1:12) Aligned. Score: 94	Sequences (4:16) Aligned. Score: 95	Sequences (9:14) Aligned. Score: 97
Sequences (1:13) Aligned. Score: 95	Sequences (5:6) Aligned. Score: 97	Sequences (9:15) Aligned. Score: 97
Sequences (1:14) Aligned. Score: 95	Sequences (5:7) Aligned. Score: 97	Sequences (9:16) Aligned. Score: 97
Sequences (1:15) Aligned. Score: 94	Sequences (5:8) Aligned. Score: 97	Sequences (10:11) Aligned. Score: 97
Sequences (1:16) Aligned. Score: 95	Sequences (5:9) Aligned. Score: 96	Sequences (10:12) Aligned. Score: 98
Sequences (2:3) Aligned. Score: 97	Sequences (5:10) Aligned. Score: 96	Sequences (10:13) Aligned. Score: 97
Sequences (2:4) Aligned. Score: 96	Sequences (5:11) Aligned. Score: 96	Sequences (10:14) Aligned. Score: 97
Sequences (2:5) Aligned. Score: 95	Sequences (5:12) Aligned. Score: 97	Sequences (10:15) Aligned. Score: 97
Sequences (2:6) Aligned. Score: 95	Sequences (5:13) Aligned. Score: 96	Sequences (10:16) Aligned. Score: 97
Sequences (2:7) Aligned. Score: 95	Sequences (5:14) Aligned. Score: 97	Sequences (11:12) Aligned. Score: 98
Sequences (2:8) Aligned. Score: 95	Sequences (5:15) Aligned. Score: 96	Sequences (11:13) Aligned. Score: 96
Sequences (2:9) Aligned. Score: 94	Sequences (5:16) Aligned. Score: 96	Sequences (11:14) Aligned. Score: 97
Sequences (2:10) Aligned. Score: 95	Sequences (6:7) Aligned. Score: 99	Sequences (11:15) Aligned. Score: 96
Sequences (2:11) Aligned. Score: 95	Sequences (6:8) Aligned. Score: 97	Sequences (11:16) Aligned. Score: 97
Sequences (2:12) Aligned. Score: 95	Sequences (6:9) Aligned. Score: 96	Sequences (12:13) Aligned. Score: 96
Sequences (2:13) Aligned. Score: 95	Sequences (6:10) Aligned. Score: 96	Sequences (12:14) Aligned. Score: 97
Sequences (2:14) Aligned. Score: 95	Sequences (6:11) Aligned. Score: 97	Sequences (12:15) Aligned. Score: 97
Sequences (2:15) Aligned. Score: 95	Sequences (6:12) Aligned. Score: 97	Sequences (12:16) Aligned. Score: 97
Sequences (2:16) Aligned. Score: 95	Sequences (6:13) Aligned. Score: 96	Sequences (13:14) Aligned. Score: 97
Sequences (3:4) Aligned. Score: 97	Sequences (6:14) Aligned. Score: 97	Sequences (13:15) Aligned. Score: 97
Sequences (3:5) Aligned. Score: 96	Sequences (6:15) Aligned. Score: 96	Sequences (13:16) Aligned. Score: 97
Sequences (3:6) Aligned. Score: 96	Sequences (6:16) Aligned. Score: 97	Sequences (14:15) Aligned. Score: 97
Sequences (3:7) Aligned. Score: 95	Sequences (7:8) Aligned. Score: 96	Sequences (14:16) Aligned. Score: 97
Sequences (3:8) Aligned. Score: 95	Sequences (7:9) Aligned. Score: 96	Sequences (15:16) Aligned. Score: 97
Sequences (3:9) Aligned. Score: 95	Sequences (7:10) Aligned. Score: 96	
Sequences (3:10) Aligned. Score: 95	Sequences (7:11) Aligned. Score: 96	
Sequences (3:11) Aligned. Score: 95	Sequences (7:12) Aligned. Score: 96	
Sequences (3:12) Aligned. Score: 96	Sequences (7:13) Aligned. Score: 96	
Sequences (3:13) Aligned. Score: 95	Sequences (7:14) Aligned. Score: 96	
Sequences (3:14) Aligned. Score: 96	Sequences (7:15) Aligned. Score: 95	
Sequences (3:15) Aligned. Score: 95	Sequences (7:16) Aligned. Score: 97	
Sequences (3:16) Aligned. Score: 95	Sequences (8:9) Aligned. Score: 97	
Sequences (4:5) Aligned. Score: 96	Sequences (8:10) Aligned. Score: 97	

## The *matK* sequence of *S. thorelii*, *S. nux-vomica*, *S. lucida* and *S. nux-blanda*

>*matK*-St1

ATGGAGGAAATCCAAGATATTTACAGCTTGATAGATCTCAACAACACGGCTTTCTATATCAACACTTCTCTTTCAAGAAGAGTATATTTATGCACTTGCTCA  
 TGATCATAGCTTAAAAACCGATCTATTTTGTGGAAAGGGAAAAATCCAGGTTATGATAATAAATCCAGGTTAGTAGGTAATAATTGTGAAACGTTTTCTAGGACT  
 GGGTAATTACTGTTAATTACGCGAATGTATCAACAGTTAAATCATTTTTATGATACAAGGTTTTCTAATAATTCTAAGCAAAATCGATTTTTATTTTGGG  
 GCACAGCAAGAATTTGTATTCTCAATGATATCAGAGGGATTTCTTTATTGTGGAATTCGTTTTCTATACGGATTACTATCTTCTCTATAGAAGAAGAGG  
 AAAGGGGATTCAAATTACAGAATTTACATTTACGCTCAATTCATTCAATAATTCCTTTCTTAGAGGACAACTTTTCACATCTAAATATGTGTTAGATATACT  
 AATACCCACCCCGTCCATCTGGAAATCCTGGTTCAAACCTTCGCTATTGGGTAAGATGCCTCCTCTTTGCATTTATTACGATTCTTCTCTACGAGTATT  
 GTAATTATTGTAATTGGAATAATCTTATTGCTACAAAGAAACCCAGTTTTCTTTTTACCAAAAAGAAATAAAGATTATTCTTCTCTATAAAAATCTTATG  
 TATGTGAATACGAATCCATTTGCTATTACTACATAACCAATCTTCTCATTACGATCAACATCCTTTGAGGTCCTCTTGAACGAATCTATTTCTATGAAAA  
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 GGTTCAAAAGGGACGGCTCCTTTGATGAATAAATGGAATCTTACCTTGCAATTTTGGCAATGTCATTTGACCTGGGGTTTCACTCCGGGAAGGGTCTA  
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 AATTAATTCATTTCCTAATCCAATAATTCCTATTTAGGAAACAATTTGGGAAACCGTTTTGTTTCAAAAATATCCCCCTGGGGTGGGGAACATTGTGGGTT  
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 GAGTTTCTTCCCTTTGGGGGGTGTAGAGAAGTCGGTTTTAATATTGGTATTGAATTTATCACTGATCTGGTGAATCATCAATGATTATCATTTACTCTG  
 AGACCTTGTAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTGAAATGTTGATGTAGCATGTAATAAGGGTTAAATCA  
 ACTGACTATTCTGCTTCTAAATGAGGCTCAAAAAAAGAAA

>*matK*-St2

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 GTGTAATTACTGTTAATTACGCGAATGTATCAACAGTTAAATCATTTTTATGATACAAGGTTTTCTAATGATTCTAAGCAAAATCGATTTTTATTTTGGG  
 GCACAGCAAGAATTTGTATTCTCAATGATATCAGAGGGATTTCTTTATTGTGGAATTCGTTTTTAAACGGATTACTATCTTCTCTATAGAAGAAGAGG  
 AAAGGGGATTCAAATTACAGAATTTACATTTACGCTCAATTCATTCAATATTTCTTTCTTAGAGGACAACTTTTCACATCTAAATATGTGTTAGATATACT  
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 GTAATTATTGTAATTGGAATAATCTTATTGCTACAAAGAAACCCAGTTTTCTTTTTACCAAAAAGAAATAAAGATTATTCTTCTCTATATAAATCTTATG  
 TATGTGAATACGAATCCATTTCTCTTTCTACATAACCAATCTTCTCATTACGATCAACATCCTTTGAGGTCCTCTTGAACGAATCTATTTCTATGAAAA  
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 GAGACCTTGTAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTGAAATGTTGATGTAGCATGTAATAAGGGTTAAATCA  
 AACTGACTATTCTGCTTCTAAATTAAGTCTAAAAAAGAAA

>*matK*-St3

ATGGAGGAAATCCAAGATATTTACAGCTTGATAGATCTCAACAACACGGCTTTCTATATCAACACTTCTCTTTCAAGAAGAGTATATTTATGCACTTGCTCA  
 TGATCATAGCTTAAAAACCGATCTATTTTGTGGAAAGGGAAAAATCGACATAAAGGGTCGAGGTTAGTAGGATCCCTACTAAATCGTTTTCTAGGAC  
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>matK-Sb1

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>matK-Sb2

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>matK-Sb3

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>matK-Sb4

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 AGACCTTGTAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTGAAATGTTGATGTAGCATGTAATAAGGGTTAAATCA  
 ACTGACTATTCTGCTTTTTGAAATAAAGTCTTAAAAGGAAACA

## The multiple sequence alignment of *matK* gene

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matK-Sv3      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGGGCTTCTATA 60
matK-Sv5      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGGGCTTCTATA 60
matK-Sv2      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGGGCTTCTATA 60
matK-Sv4      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGGGCTTCTATA 60
matK-Sv1      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGGGCTTCTATA 60
matK-Sl2      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-Sl3      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-s11      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-Sb1      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-Sb2      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-Sb3      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-Sb4      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-St1      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-St4      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-St3      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
matK-St2      ATGGAGGAAATCCAAAGATATTTACAGCTTGATAGATCTCAACAACACGG-CTTCTATA 59
*****

matK-Sv3      TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTACTCATGATCATAGTTTAAA 119
matK-Sv5      TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGTTTAAA 119
matK-Sv2      TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGTTTAAA 119
matK-Sv4      TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGTTTAAA 119
matK-Sv1      TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGCTTAAA 119
matK-Sl2      TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGCTTAAA 118
matK-Sl3      TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGCTTAAA 118
matK-s11      TCAACACTTCTCTTTCAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGCTTAAA 118
matK-Sb1      TCAACTTCTCTTTCAACAAGAGTATATTT-ATCCACTAECTCATGATCATAGCTTAAA 118
matK-Sb2      TCAACTTCTCTTTCAACAAGAGTATATTT-ATCCACTAECTCATGATCATAGCTTAAA 118
matK-Sb3      CCAACTTCTCTTTCAACAAGAGTATATTT-ATCCACTAECTCATGATCATAGCTTAAA 118
matK-Sb4      TCAACACGTTTCTCTAAAGAAGAGTATATTT-ATGCATTTGCTCATGATCATAGCTTAAA 118
matK-St1      TCAACACTTCTCTTTCAAGAAGAGTATATTTATGCACCTGCTCATGATCATAGCTTAAA 119
matK-St4      TCAACACTTCTCTTTCAAGAAGAGTATATTTATGCACCTGCTCATGATCATAGCTTAAA 119
matK-St3      TCAACACTTCTCTTTCAAGAAGAGTATATTTATGCACCTGCTCATGATCATAGCTTAAA 119
matK-St2      TCAACACTTCTCTTTCAAGAAGAGTATATTTATGCACCTGCTCATGATCATAGCTTAAA 119
****          * * *          *****          *****          * * *          *****          *****

matK-Sv3      AACCGATCTATTTTGGTGGGAAGGAAAAGCCAGGCT-TTTTTGGAAAATCC-AGGGTAAT 177
matK-Sv5      AACCGGTCATTTTGGTGGGAAGGAAAATCCAAAGGT-TTTTTGGAAAATCC-AGGGTAAT 177
matK-Sv2      AACCGATCTATTTTGGTGGGAATGGAAAACCCATGCT-TTTTTGGACAATCC-AGGGTAAT 177
matK-Sv4      AACCGGTCATTTTGGTGGGAATGGAAAATCCATGGT-TTTTTGGACAATCC-AGGGTAAT 177
matK-Sv1      AACCGATCTATTTTGGTGGGAAGGAAAATCCAGGTT-TTGTGGACAATCC-AGGGTAGT 177
matK-Sl2      AACCGATCTATATTTGGTGGACGGGAATTTCCAGATT-TTGTGGACAACCC-AGGTTAGT 176
matK-Sl3      AACCGATCTATATTTGGTGGACGGGAATTTCCAGATT-TTGTGGACAACCC-AGGTTAGT 176
matK-s11      AACCGATCTATTTTGGTGGGAAGGAAAATCCAGGTT-TTGTGGACAATCC-AGGTTAGT 176
matK-Sb1      AAGCGATCTATTTTGGTGAACCGAAAATCCCGGT--GTCTGAGAAAATCTCAGGTTAAA 176
matK-Sb2      AATCGATCTATTTTGGTGAAGGAAAATCCCGGT--GTCTGAGAAAATCTCAGGTTAAA 176
matK-Sb3      AACCGATCTATTTTGGTGAAGGAAAATCCCGGTTAACCTAGTGAAACC--GGTTAGT 176
matK-Sb4      AACCGATCTATTTTGGTGAAGGAAAATCCCGGTT-TTGTGGAAAATCC-AGGTTAGT 176
matK-St1      AACCGATCTATTTTGGTGGGAAGGAAAATCCAGGTT-ATGATAATAAAATCC-AGGTTAGT 177
matK-St4      AACCGATCTATTTTGGTGGGAAGGAAAATCCACCTC-ATGATAATAAAATCC-AGGTTAGT 177
matK-St3      AACCGATCTATTTTGGTGGGAAGGAAAATCCAGGTT-ATGATGGAAAATCC-AGGTTAGT 177
matK-St2      AACCGATCTATTTTGGTGGGAAGGAAAATCCAGGTT-ATGATGGAAAATCC-AGGTTAGT 177
**          *          *****          * * *          *          *          *****          * * *

matK-Sv3      CGATAATAAGATCAAAACGTTATCTAAGGTGGTGAACACTAGTTTTTAATTACTCAAATGT 237
matK-Sv5      CGATAAAAAGATCAAAACGTTATCTAAGGTGGTGAACACTAGTTTTTAATTACTCAAATGT 237
matK-Sv2      CGATAATAAGATCAAAACGTTATCTAAGGTGGTGAACACTACTTTTTAATTACTCAAATGT 237
matK-Sv4      CGATAATAAGATCAAAACGTTATCTAAGGTGGTGAACACTAGTTTTTAATTACTCAAATGT 237
matK-Sv1      CGATAATAAGATCAAAACGTTATCTAAGGTGGTGAACACTACTTTTTAATTACTCAAATGT 237
matK-Sl2      TGATAATAAATCCAAAACGTTTTCTAAGATTGTGAAAATCCCGTGTAAATTACTCGAATGT 236
matK-Sl3      TGATAATAAATCCAAAACGTTTTCTAAGATTGTGAAAATCCCGTGTAAATTACTCGAATGT 236
matK-s11      TGATAATAAATCCAAAACGTTTTCTAGGATTGTGAAAACACTACTTTTTAATTACTCGAATGT 236
matK-Sb1      TGATAATAAATCCAAAACGTTTTCTAGGATTGTGAAAACACTACTGTTTAATTACTCGAATGT 236
matK-Sb2      TGATAATAAATCCAAAACGTTTTCTAGGATTGTGAAAACACTACTGTTTAATTACTCGAATGT 236
matK-Sb3      TGATAATAAATCCAAAACGTTTTCTAGGATTGTGAAAACACTACTGTTTAATTACTCGAATGT 236
matK-Sb4      TGATAATAAATCCAAAACGTTTTCTAGGATTGTGAAAACACTACTGTTTAATTACTCGAATGT 236
matK-St1      AGGTAATAATTGTGAAAACGTTTTCTAGGACTGGGTAATTACTGTTTAATTACTCGAATGT 237
matK-St4      AGGTAACCTAGTAAAACGTTTTCTAGGAGTGGGTAATAACTGTTTAATTACTCGAATGT 237
matK-St3      AGGTAATCCCTACTAAAACGTTTTCTAGGACTGGGTAATAACTGTTTAATTACTCGAATGT 237
matK-St2      AGATACTAATTGTAAAACGTTTTCTAGGACTGTGTAATTACTGTTTAATTACTCGAATGT 237
* * *          **          *****          * * *          * * *          *****          * * *

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*matK-Sv3* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1736  
*matK-Sv5* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1736  
*matK-Sv2* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1736  
*matK-Sv4* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1736  
*matK-Sv1* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1736  
*matK-Sl2* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1731  
*matK-Sl3* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1731  
*matK-sl1* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1731  
*matK-Sb1* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1730  
*matK-Sb2* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1730  
*matK-Sb3* AAATGGATTTAAGCCTAAACTAAAATGATGAAGATGAAGAAAAAGTTTTCTACTATTCTG 1730  
*matK-Sb4* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1730  
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*matK-St2* AAATGGATTTAAGCCTAAATGAAAATGATGAAGAGATAACAAAAGTTTTCTACTATTCTG 1731  
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*matK-Sv3* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTCTAAATA 1796  
*matK-Sv5* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTCTAAATA 1796  
*matK-Sv2* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTCTAAATA 1796  
*matK-Sv4* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTCTAAATA 1796  
*matK-Sv1* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTCTAAATA 1796  
*matK-Sl2* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTCTAAACTA 1791  
*matK-Sl3* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTCTAAACTA 1791  
*matK-sl1* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTCTAAACTA 1791  
*matK-Sb1* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTAAATTA 1790  
*matK-Sb2* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTAAACTA 1790  
*matK-Sb3* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTAAATTA 1790  
*matK-Sb4* AAATGTTGATGTAGCATGTAATAAGGGTTAAATCAACTGACTATTCTGCCTTTTGAATA 1790  
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*matK-Sv3* AAGTCTAAAAAAGGAAAA 1815  
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*matK-Sv4* AAGTCTAAAAAAGGAAAA 1815  
*matK-Sv1* AAGTCTAAAAAAGGAAAA 1815  
*matK-Sl2* AAGTTTAAATGAGTTATCA 1810  
*matK-Sl3* AAGTTTTAATGAGTTATCC 1810  
*matK-sl1* AAGTCTAGATGAGTTATCA 1810  
*matK-Sb1* AAGTCTTTAAAAGGAAACA 1809  
*matK-Sb2* AAGTCTTTAAAAGGAAACA 1809  
*matK-Sb3* AAATCTTTAAAAGGAAACA 1809  
*matK-Sb4* AAGTCTTTAAAAGGAAACA 1809  
*matK-St1* AGGTCTAAAAAAAAGAAA 1810  
*matK-St4* AAGTTTAAAAAAAAGAAA 1810  
*matK-St3* AAGTTTAAAAAAAAGAAA 1808  
*matK-St2* AAGTCTAAAAAAAAGAAA 1810  
 \* \* \* \* \*

**The size of the *matK* gene**

Sequence 1: <i>matK</i> -St1	1810 bp
Sequence 2: <i>matK</i> -St2	1810 bp
Sequence 3: <i>matK</i> -St3	1808 bp
Sequence 4: <i>matK</i> -St4	1810 bp
Sequence 5: <i>matK</i> -sl1	1810 bp
Sequence 6: <i>matK</i> -Sl2	1810 bp
Sequence 7: <i>matK</i> -Sl3	1810 bp
Sequence 8: <i>matK</i> -Sv1	1815 bp
Sequence 9: <i>matK</i> -Sv2	1815 bp
Sequence 10: <i>matK</i> -Sv3	1815 bp
Sequence 11: <i>matK</i> -Sv4	1815 bp
Sequence 12: <i>matK</i> -Sv5	1815 bp
Sequence 13: <i>matK</i> -Sb1	1809 bp
Sequence 14: <i>matK</i> -Sb2	1809 bp
Sequence 15: <i>matK</i> -Sb3	1809 bp
Sequence 16: <i>matK</i> -Sb4	1809 bp



### The pairwise of similarity score

Sequences (1:2) Aligned. Score: 96	Sequences (4:7) Aligned. Score: 92	Sequences (8:13) Aligned. Score: 92
Sequences (1:3) Aligned. Score: 96	Sequences (4:8) Aligned. Score: 92	Sequences (8:14) Aligned. Score: 93
Sequences (1:4) Aligned. Score: 96	Sequences (4:9) Aligned. Score: 92	Sequences (8:15) Aligned. Score: 92
Sequences (1:5) Aligned. Score: 92	Sequences (4:10) Aligned. Score: 91	Sequences (8:16) Aligned. Score: 94
Sequences (1:6) Aligned. Score: 91	Sequences (4:11) Aligned. Score: 92	Sequences (9:10) Aligned. Score: 99
Sequences (1:7) Aligned. Score: 91	Sequences (4:12) Aligned. Score: 91	Sequences (9:11) Aligned. Score: 99
Sequences (1:8) Aligned. Score: 91	Sequences (4:13) Aligned. Score: 92	Sequences (9:12) Aligned. Score: 98
Sequences (1:9) Aligned. Score: 91	Sequences (4:14) Aligned. Score: 92	Sequences (9:13) Aligned. Score: 92
Sequences (1:10) Aligned. Score: 90	Sequences (4:15) Aligned. Score: 92	Sequences (9:14) Aligned. Score: 93
Sequences (1:11) Aligned. Score: 90	Sequences (4:16) Aligned. Score: 93	Sequences (9:15) Aligned. Score: 92
Sequences (1:12) Aligned. Score: 90	Sequences (5:6) Aligned. Score: 97	Sequences (9:16) Aligned. Score: 94
Sequences (1:13) Aligned. Score: 91	Sequences (5:7) Aligned. Score: 98	Sequences (10:11) Aligned. Score: 99
Sequences (1:14) Aligned. Score: 91	Sequences (5:8) Aligned. Score: 94	Sequences (10:12) Aligned. Score: 98
Sequences (1:15) Aligned. Score: 91	Sequences (5:9) Aligned. Score: 94	Sequences (10:13) Aligned. Score: 92
Sequences (1:16) Aligned. Score: 92	Sequences (5:10) Aligned. Score: 94	Sequences (10:14) Aligned. Score: 92
Sequences (2:3) Aligned. Score: 96	Sequences (5:11) Aligned. Score: 94	Sequences (10:15) Aligned. Score: 92
Sequences (2:4) Aligned. Score: 96	Sequences (5:12) Aligned. Score: 93	Sequences (10:16) Aligned. Score: 93
Sequences (2:5) Aligned. Score: 95	Sequences (5:13) Aligned. Score: 93	Sequences (11:12) Aligned. Score: 99
Sequences (2:6) Aligned. Score: 93	Sequences (5:14) Aligned. Score: 94	Sequences (11:13) Aligned. Score: 92
Sequences (2:7) Aligned. Score: 93	Sequences (5:15) Aligned. Score: 93	Sequences (11:14) Aligned. Score: 92
Sequences (2:8) Aligned. Score: 93	Sequences (5:16) Aligned. Score: 95	Sequences (11:15) Aligned. Score: 92
Sequences (2:9) Aligned. Score: 92	Sequences (6:7) Aligned. Score: 99	Sequences (11:16) Aligned. Score: 94
Sequences (2:10) Aligned. Score: 92	Sequences (6:8) Aligned. Score: 93	Sequences (12:13) Aligned. Score: 92
Sequences (2:11) Aligned. Score: 92	Sequences (6:9) Aligned. Score: 92	Sequences (12:14) Aligned. Score: 92
Sequences (2:12) Aligned. Score: 92	Sequences (6:10) Aligned. Score: 92	Sequences (12:15) Aligned. Score: 91
Sequences (2:13) Aligned. Score: 94	Sequences (6:11) Aligned. Score: 92	Sequences (12:16) Aligned. Score: 93
Sequences (2:14) Aligned. Score: 94	Sequences (6:12) Aligned. Score: 92	Sequences (13:14) Aligned. Score: 98
Sequences (2:15) Aligned. Score: 94	Sequences (6:13) Aligned. Score: 92	Sequences (13:15) Aligned. Score: 97
Sequences (2:16) Aligned. Score: 95	Sequences (6:14) Aligned. Score: 93	Sequences (13:16) Aligned. Score: 97
Sequences (3:4) Aligned. Score: 97	Sequences (6:15) Aligned. Score: 92	Sequences (14:15) Aligned. Score: 98
Sequences (3:5) Aligned. Score: 93	Sequences (6:16) Aligned. Score: 94	Sequences (14:16) Aligned. Score: 97
Sequences (3:6) Aligned. Score: 92	Sequences (7:8) Aligned. Score: 93	Sequences (15:16) Aligned. Score: 96
Sequences (3:7) Aligned. Score: 92	Sequences (7:9) Aligned. Score: 92	
Sequences (3:8) Aligned. Score: 92	Sequences (7:10) Aligned. Score: 92	
Sequences (3:9) Aligned. Score: 92	Sequences (7:11) Aligned. Score: 92	
Sequences (3:10) Aligned. Score: 91	Sequences (7:12) Aligned. Score: 92	
Sequences (3:11) Aligned. Score: 91	Sequences (7:13) Aligned. Score: 92	
Sequences (3:12) Aligned. Score: 91	Sequences (7:14) Aligned. Score: 93	
Sequences (3:13) Aligned. Score: 92	Sequences (7:15) Aligned. Score: 93	
Sequences (3:14) Aligned. Score: 92	Sequences (7:16) Aligned. Score: 94	
Sequences (3:15) Aligned. Score: 92	Sequences (8:9) Aligned. Score: 98	
Sequences (3:16) Aligned. Score: 93	Sequences (8:10) Aligned. Score: 98	
Sequences (4:5) Aligned. Score: 93	Sequences (8:11) Aligned. Score: 98	
Sequences (4:6) Aligned. Score: 92	Sequences (8:12) Aligned. Score: 98	



## VITA

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### Publication

Nakkiang, K., Palanuvej, C., and Ruangrunsi, R. Microscopic analysis of Selected Strychnos species in Thailand. Proceedings of the 2nd International Conference on Advanced Pharmaceutical Research Strategies and Innovation in Pharmaceutical Research: Safety, Efficacy and Quality, pp. 164-169, Pathumthani, 2015.





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