วิวัฒนาการร่วมของยืนโทโพไอโซเมอเรส I กับการสร้างแคมป์โทเธซินในพืชสกุล Ophiorrhiza

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต สาขาวิชาเภสัชเวท ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปีการศึกษา 2553 ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

COEVOLUTION OF *TOPOISOMERASE* I AND CAMPTOTHECIN PRODUCTION IN *OPHIORRHIZA* PLANTS

Miss Varalee Viraporn

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacy Program in Pharmacognosy Department of Pharmacognosy and Pharmaceutical Botany Faculty of Pharmaceutical Sciences Chulalongkorn University Academic Year 2010 Copyright of Chulalongkorn University

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แคมป์โทเธซินเป็นสารในกลุ่ม indole alkaloid ซึ่งพบในธรรมชาติ และเป็นสารตั้งต้น ในกระบวนการผลิตยาเคมีบำบัดที่มีใช้กันอย่างแพร่หลายทั่วโลก ความต้องการในการใช้ยา ปัจจุบันแคมป์โทเธซินยังคงได้มาจากการสกัดจากพืช ได้แก่ กลุ่มนี้มีเพิ่มมากขึ้น Camptotheca acuminata และ Nothapodytes foetida มีรายงานว่าพืชสกุล Ophiorrhiza บางชนิดสามารถสร้างแคมป์โทเธซิน และพบว่าพืชหลายชนิดที่สร้างแคมป์โทเธซินมีเจนไซม์ โทโพไอโซเมอเรล I ที่เกิดการกลายพันธุ์ในหลายตำแหน่ง ทำให้แคมป์โทเธซินไม่สามารถเข้า จับกับเอนไซม์ โทโพไอโซเมอเวล I ได้ ส่งผลให้พืชเหล่านั้นทนทานต่อพิษของแคมป์โทเธซินที่ พืชสร้างขึ้นมาเอง งานวิจัยนี้เป็นการศึกษาวิวัฒนาการร่วมของยืนโทโพไอโซเมอเรส I กับการ สร้างแคมป์โทเธซินของพืชสกุล Ophiorrhiza 8 ชนิดในประเทศไทย พบว่ามีพืช 5 ชนิดที่สร้าง แคมป์โทเธซินและอนูพันธ์ของแคมป์โทเธซินในบริเวณใบหรือราก เมื่อศึกษาลำดับนิวคลีโอ ไทด์ของของยีนแม็ตเคและยีน<mark>โทโพไอโซเมอเรส I</mark> ของพืชสกุล Ophiorrhiza เพื่อจัดจำแนก กลุ่มพืชและศึกษาวิวัฒนาการ พบว่าวงศ์วานวิวัฒนาการเชิงโมเลกุลของยืนแม็ตเค, ยืนโทโพ ไอโซเมอเรส I, และทั้งสองยืนสามารถแบ่งกลุ่มพืช Ophiorrhiza เป็นสองกลุ่มตามคุณสมบัติ ในการสร้างแคมป์โทเธซินและอนุพันธ์ของแคมป์โทเธซิน สรุปว่าพันธุกรรมมีบทบาทสำคัญ ในการกำหนดความสามารถในการสร้างสารกลุ่มแคมป์โทเธซินในพืชสกุลนี้ และพืชสกุล Ophiorrhiza มีวิวัฒนาการร่วมระหว่างยืนแม็ตเคและยืนโทโพไอโซเมอเรล I กับการสร้าง แคมป์โทเธซินและอนุพันธ์ ดังนั้นจึงสามารถใช้ยืนแม็ตเคและยืนโทโพไอโซเมอเรล เ เพื่อช่วย ในการทำนายการสร้างแคมป์โทเธซินและอนุพันธ์ของแคมป์โทเธซินของพืชในสกุล Ophiorrhiza ได้ นอกจากนั้นยังพบการแทนที่ของกรดอะมิโนหลายตำแหน่งในเอนไซม์โทโพ ไอโซเมอเรล I ที่สามารถใช้เป็นเครื่องหมายในการจำแนกกลุ่มพืชที่สร้างและกลุ่มพืชที่ไม่สร้าง แคมป์โทเธซินซึ่งใช้เป็นข้อมูลพื้นฐานในการทำนายการดื้อยาในผู้ป่วยโรคมะเร็งในอนาคต

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VARALEE VIRAPORN: COEVOLUTION OF *TOPOISOMERASE* I AND CAMPTOTHECIN PRODUCTION IN *OPHIORRHIZA* PLANTS. THESIS ADVISOR: ASSISTANT PROFESSOR SUCHADA SUKRONG, Ph.D., THESIS CO-ADVISOR: TAKSINA CHUANASA, Ph.D., 124 pp.

Camptothecin (CPT), a naturally occurring indole alkaloid, is an essential precursor of semi-synthetic chemotherapeutic agents for cancers throughout the world. In spite of the rapid growth of market demand, CPT raw material is still harvested by extraction from Camptotheca acuminata and Nothapodytes foetida. Previous study found that many CPT-producing plants, including some of Ophiorrhiza spp., have topoisomerase I (TopI) enzymes with several point-mutations that confer resistance to CPT to avoid CPT toxicity. The purpose of this thesis is to study the coevolution between Topl gene and CPT production in Ophiorrhiza plants. Eight species of the genus Ophiorrhiza in Thailand were examined as novel alternative sources of CPT. CPT and its derivatives were differently detected in five species in leaf and root extracts. Chloroplast matK and nuclear Topl genes of eight species were investigated in order to classify and study the coevolution in this genus. The molecular phylogenetic trees of both separated and combined matk and Topl nucleotide sequences revealed two major clades of Ophiorrhiza taxa correlated with the productions of CPT and CPT derivatives. We conclude that Ophiorrhiza plants have matK and Topl coevolved with CPT production. Thus, matK and TopI gene sequences could be utilized for the prediction of CPT and CPT derivatives production ability of any members of Ophiorrhiza. We also proposed that several unique amino acid substitutions in Topl of CPT-producing Ophiorrhiza plants could be used as amino acid markers and provide useful information toward recognition of the point mutations in CPT-resistant cancer patients in the future.

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LIST OF ABBREVIATIONS

%	= percent (part per 100); percentage
A, T, C, G	= nucleotide containing the base adenine, thymine, cytosine, and
	guanine, respectively
bp	= base pair
cDNA	= complementary deoxyribonucleic acid
CI	= consistency index
°C	= degree celsius
DMF	= dimethylformamide
DNA	= deoxyribonucleic acid
dNTPs	= deoxyribonucleotide triphosphates (dATP, dTTP, dGTP, dCTP)
g	= gram(s)
HOAc	= acetic acid
HPLC-DAD	= high-performance liquid chromatography-diode array detection
hr	= hour(s)
HRMS	= high resolution mass spectrometry
H ₂ O	= water
ITS	= internal transcribed spacer
kb	= kilobase
kDa	= kilodalton
L	= liter(s)
LB-Amp	= lysogeny broth containing the antibiotic ampicillin
LC-MS/MS	= liquid chromatography-mass spectrometry/ mass spectrometry
Μ	= molar
MeOH	= methanol
MgCl ₂	= magnesium chloride
min	= minute(s)
mL	= milliliter
mM	= millimolar

MPT(s)	= maximum parsimonious tree(s)
Ν	= northern
NE	= north eastern
ng	= nanogram(s)
nm	= nanometer
PAUP	= phylogenetic analysis using parsimony
PCR	= polymerase chain reaction
RC	= rescaled consistency index
RI	= retention index
рН	= the negative logarithm of the concentration of hydrogen ions
RNA	= ribonucleic acid
rRNA	= ribosomal ribonucleic acid
rpm	= revolution per minute
S	= second(s)
SE	= south eastern
SW	= south western
SD	= standard deviation
sp.	= species (singular)
spp.	= species (plural)
TAE buffer	= tris-acetate and EDTA buffer
TIA(s)	= monoterpenoid indole-alkaloid(s)
Trp	= tryptophan (amino acid)
UV	= ultraviolet
μg	= microgram(s)
μL	= microliter(s)
μΜ	= micromolar
WS	= west southern

Amino acid abbreviations

А	/ Ala	= alanine
С	/ Cys	= cysteine
D	/ Asp	= aspartic acid
Е	/ Glu	= glutamic acid
F	/ Phe	= phenylalanine
G	/ Gly	= glycine
Н	/ His	= histidine
I	/ lle	= isoleucine
Κ	/ Lys	= lysine
L	/ Leu	= leucine
Μ	/ Met	= methionine
Ν	/ Asn	= asparagine
Ρ	/ Pro	= proline
Q	/ Gln	= glutamine
R	/ Arg	= arginine
S	/ Ser	= serine
Т	/ Thr	= threonine
V	/ Val	= valine
W	/ Trp	= tryptophan
Y	/ Tyr	= tyrosine

CHAPTER I

INTRODUCTION

Camptothecin (CPT), a naturally occurring pentacyclic indole alkaloid, exhibits an anticancer activity due to its ability to inhibit topoisomerase I (TopI) enzyme involving in DNA topology (Hsiang et al., 1985). Knowledge of the structure-activity relationship of CPT has led to the development of CPT derivatives to increase solubility, stability, and bioavailability with manageable toxicities (Dancey and Eisenhauer, 1996). Two semisynthetic CPT analogs, topotecan (Hycamtin[®]) and irinotecan (Camptosar[®]) are currently used throughout the world for various cancer treatments, such as ovarian cancer, lung cancer, colon cancer, and over a dozen more CPT analogs are at various stages of clinical development (Lorence and Nessler, 2004). In spite of the rapid growth of market demand, CPT raw material is still harvested by extraction from Camptotheca acuminata and Nothapodytes foetida since its total synthesis is not cost effective. As a result, this could lead to a lack or an extinction of CPT-producing plants in the future. Plants reported to contain CPT are C. acuminata (Nyssaceae) (Wall et al., 1966), N. foetida (Icacinaceae) (Govindachari and Viswanathan, 1972), Ervatamia heyneana (Apocynaceae) (Gunasekera et al., 1979), and some species in the genus Ophiorrhiza (Rubiaceae) (Lorence and Nessler, 2004). Recently, CPT-producing Ophiorrhiza plants have become interesting as alternative sources for CPT production in tissue cultures (Martin et al., 2008; Roja, 2008).

Ophiorrhiza L. is a predominantly herbaceous genus belonging to the family Rubiaceae and comprising about 400 species (Schanzer, 2005). Since the genus is taxonomically complex and has high morphological variability (Chou, Yang, and Liao 2006; Darwin, 1976; Kudoh *et al.*, 2001), few studies have attempted to resolve this taxonomic problem using molecular phylogenetic systematics (Nakamura *et al.*, 2006, 2007). Several DNA regions, such as ITS, *atp*B-*rbc*L and *trnK/mat*K have been utilized to determine the species and varieties within *Ophiorrhiza*. Previous study found that many CPT-producing plants including *Camptotheca acuminata*, *N. foetida*, *O. pumila* and *O.* *liukiuensis* have Topl enzymes with several point-mutations that confer resistance to CPT to avoid CPT toxicity. This could be inferred as a self-resistance mechanism coevolved with the production of CPT (Sirikantaramas, Yamazaki, and Saito, 2008).

This thesis aims to study the coevolution between *Topl* gene and CPT production in *Ophiorrhiza* plants. The research consists of two main parts. The first part is to study CPT-producing ability of *Ophiorrhiza* plants. The methanol extracts of *Ophiorrhiza* species were analyzed for CPT compound using HPLC/DAD/ESI/MS. Standard solutions of CPT, CPT derivatives, and other chemical compounds involved in CPT biosynthesis pathway were also analyzed using the same method. In the second part, the molecular phylogenetic trees of chloroplast *mat*K and nuclear *Topl* nucleotide sequences were reconstructed to classify and study an evolution in this genus. Furthermore, amino acid sequences of Topl enzymes were analyzed to detect point mutations in the CPT-producing *Ophiorrhiza* spp. In conclusion, the molecular phylogenetic trees and the CPT-producing ability were concurrently analyzed to define the coevolution of *Topl* and CPT production in *Ophiorrhiza* plants.

In this study, five out of eight species of *Ophiorrhiza* were discovered as novel alternative sources of CPT and CPT derivatives. We found that both *mat*K and *Top*I of *Ophiorrhiza* plants coevolved with CPT production. Thus, *mat*K and *Top*I gene sequences could be utilized for the prediction of CPT- and CPT derivatives-producing ability of members of *Ophiorrhiza*. We also proposed that several unique amino acid substitutions in TopI of CPT-producing *Ophiorrhiza* plants could be used as useful markers and provide information toward recognition of the point mutations in CPT-resistant cancer patients in the future.

CHAPTER II

LITERATURE REVIEW

2.1 Camptothecin (CPT)

2.1.1 Structure and biosynthesis pathway of CPT

CPT (Figure 2.1) is a plant-originated pentacyclic indole alkaloid (Yamazaki et al., 2004). The pentacyclic ring system includes a pyztolo[3,4-b]quinoline (ring A, B and C), a conjugated pyridine (ring D), and six-membered lactone (ring E) with a chiral center at position C-20 (Narkunan et al., 2009). The structural features of CPT that are essential for activity include the 20(S)-hydroxyl (Wang, Zhou, and Hecht, 1999), the pyridone moiety, the lactone, and the planarity of the five-membered ring system (Carbonero and Supko, 2002). Since CPT is a weak acid, the lactone ring is highly susceptible to ring opening by hydrolysis, forming carboxylate (Figure 2.2). At physiological pH, a labile E-ring lactone is hydrolyzed to an inactive hydroxy acid, which binds to human serum albumin. This reaction is reversible at acidic pH, as it is in cancer cell microenvironment, regenerating the active compound. Due to the unique chemistry of the CPT molecule, this physiology results in environmental conditions that may provide tumor site-specific enhancement of CPT activity (Flowers et al., 2003). However, the low aqueous solubility of CPT in the lactone form greatly limited the practical clinical utility of the drug because prohibitively large volumes of fluid have to be administered to the subject in order to provide an effective dose of the drug (Narkunan et al., 2009).

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Figure 2.1 Chemical structure of camptothecin.



Figure 2.2 The pH-dependent dynamic equilibrium between the lactone and carboxylic acid forms of camptothecin (Flowers *et al.*, 2003).

CPT is a product of secondary metabolism from monoterpenoid indole-alkaloids (TIAs) (Lu *et al.*, 2008) derived from amino acid tryptophan (Trp) and terpenoid precursors (Lorence and Nessler, 2004). Camptothecin is formed by the combination of the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway and the shikimate pathway, which involves many distinct enzymatic steps (Figure 2.3). The common intermediate, from which a variety of TIAs are formed, is strictosidine. Strictosidine is formed by the condensation of tryptophan-derivative tryptamine with the iridoid glucoside, secologanin. This condensation is catalyzed by a key enzyme, strictosidine synthase. Subsequently, intramolecular cyclization of strictosidine yields strictosamide, a penultimate precursor of camptothecin formation (Yamazaki *et al.*, 2003, 2004). 3(*S*)-pumiloside and 3(*S*)-deoxy pumiloside are thought to be biogenetic intermediates in the formation of CPT from strictosamide.



Figure 2.3 Biosynthetic pathway for TIAs in CPT-producing plants. Multiple arrows indicate multiple steps between intermediates. The enzymes involve in the pathways: DXP synthase (DXS); DXP reductoisomerase (DXR); 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (MECS); geraniol-10-hydroxylase (G10H); secologanin synthase (SLS); TSB (b-subunit of tryptophan synthase); TDC (tryptophan decarboxylase); SSS (strictosidine synthase), and 10-HGO (10-hydroxygeraniol oxidoreductase (source: Yamazaki *et al.*, 2004).

2.1.2 Anticancer CPT analogs

By the early 1970's, CPT had reach Phase I and Phase II clinical trials. Although CPT was found to possess antitumor activity, there were numerous side-effects including hemorrhagic cystitis, leucopenia and thrombocytopenia which were dose-limiting toxicities (Muggia *et al.*, 1972). In addition, CPT is extremely poor water soluble and easily hydrolysable due to the closed E-ring lactone. Thus, knowledge of the structure–activity relationship of CPT has led to the development of CPT derivatives to increase solubility, stability and bioavailability with manageable toxicities (Dancey and Eisenhauer, 1996). Originally, CPT was delivered as the sodium salt of the carboxylate to help overcome solubility issues, however, the poor efficacy created a need for new alternatives (Hsiang *et al.*, 1989). The modifications of the quinoline ring provided increased solubility, lactone stability (Chourpa *et al.*, 1998), and antitumor activity (Vladu *et al.*, 2000). Modifications to the 7, 9, 10, and 11 positions of the A-ring and B-ring, are generally well tolerated and in many cases enhance the potency of the CPT analog in both *in vivo* and *in vitro* studies (Redinbo *et al.*, 1998)

Two semi-synthetic water-soluble CPT analogs (Figure 2.4), topotecan (Hycamtin[®]) and irinotecan (Camptosar[®]) were approved for use by the USFDA in 1966, and over a dozen other CPT analogs are at various stages of clinical development (Lorence and Nessler, 2004). Topotecan gains its increase solubility and greater *in vivo* activity due to a tertiary amine at the 9-position, while irinotecan presents its improvement through the 10-hydroxyl moiety. Topotecan is currently approved for use in the USA as second-line therapy in ovarian and small cell lung cancer. Irinotecan is a pro-drug that undergoes enzymatic conversion to the biologically active metabolite 7-ethyl-10-hydroxy-CPT. Its approved indications were cancers of the lung (small cell and non-small cell), cervix, ovaries, and also colon cancer as a second-line agent. It is presently the treatment of choice when used in combination with fluoropyrimidines for patients with advanced colorectal cancer or as a single agent after failure of 5-fluorouracil-based chemotherapy (Carbonero and Supko, 2002).



Figure 2.4 Clinically used semi-synthetic CPT analogs, topotecan and irinotecan (Yamazaki *et al.*, 2004).

Hydroxy CPT and methoxy CPT are a naturally occurring CPT derivative isolated from many CPT-producing plants (Wani and Wall, 1969; Yamazaki *et al.*, 2004). 10-hydroxy CPT (10-HCPT) has been found to be more potent and less toxic than CPT (Zhang *et al.*, 1998). Many water-soluble aminoalkyl CPT analogs, including topotecan, were prepared by oxidation of CPT to 10-HCPT followed by additional modifications (Kingsbury *et al.*, 1991). Irinotecan is also a 10-HCPT analog synthesized by bonding phenolic hydroxyl group of 7-ethyl-10-HCPT with diamines (Sawada *et al.*, 1991). Besides, long-chain fatty acid esters of 10-HCPT derivatives could be useful as prodrugtype anticancer agents (Takayama *et al.*, 1998). The methoxy analog was found to be more active than CPT (Tafur *et al.*, 1976). 9-methoxy camptothecin (9-MCPT) has been reported as a starting material for a synthesis of 9-methoxy mappicine which has antiviral activity (Das and Madhusudhan, 1999).

2.1.3 Distribution of CPT and its derivatives

CPT is first isolated from extracts of *Camptotheca acuminata* (Nyssaceae), a deciduous tree native to China and Tibet (Wall *et al.*, 1966). Plants which have been reported containing CPT belong to the following unrelated orders and families: Order Cornales (Nyssaceae): *C. acuminata*: Order Celastrales (Icacinaceae): *Nothapodytes foetida* (Aiyama *et al.*, 1988), *Pyrenacantha klaineana* (Zhou *et al.*, 2000), *Merrilliodendron megacarpum* (Arisawa *et al.*, 1981): Order Gentianales (Rubiaceae): some species of the genus *Ophiorrhiza*, Family Apocynaceae: *Ervatamia heyneana* (Dai, Cardellina, and Boyd, 1999), and Family Gelsemiaceae: *Mostuea brunonis* (Gunasekera *et al.*, 1979). It is likely that the genes encoding enzymes involved in their biosynthesis evolved early during evolution. These genes were presumably not lost during evolution but might have been "switched off" during a certain period of time and "switched on" again at some later point (Wink, 2003).

The information regarding the sites of accumulation of CPT and CPT derivatives including their concentration in multiple natural sources are summarized in Table 2.1. The most abundant natural CPT derivatives are 10-HCPT and 9-MCPT. *N. foetida* was found to produce CPT by an endophyte at shake flask and bioreactor (Rehman *et al.*, 2009). 10-HCPT, 9-MCPT, and 10-methoxy camptothecin (10-MCPT) were also produced by endophytic fungi, *Fusarium solani*, isolated from CPT-producing plants (Shweta *et al.*, 2010). *C. acuminata* was detected the 5-6 fold of the CPT content in young leaves compared to mature ones (López-Meyer, Nessler, and McKnight, 1994). In fact, the immature leaves are attractive to herbivory and pathogen. Although the role of CPT as a defense chemical has not been directly tested, there are indirect lines of evidence indicating its involvement in plant defense (Lorence and Nessler, 2004). According to the previous study of *N. foetida* (Roja, 2006), an increase in the level of 9-MCPT in the mature plant suggests that the accumulation of the 9-MCPT may probably be associated with the maturation of the plant.

Species	Tissue	Sample origin	Camptothecinoids	Reference
	analyzed		(ua/a drv weiaht)	
Camptotheca acuminata	Young leaves	Texas, USA	CPT 4000–5000	López-Meyer et al.,
Decaisne	-		10-HCPT 20-30	1994
	Seeds		CPT 3000	
			10-HCPT 25	
	Bark		CPT 1800-2000	
	Deete		10-HCPT 2-90	
	ROOIS			
	Young leaves	Texas LISA	CPT 2421-3022	lietal 2002
	Old leaves	10,43,00,4	CPT 482	LI CI dI., 2002
	Young fruit		CPT 842	
	Old fruit		CPT 2362	
	Hairy roots	Texas, USA	CPT 1000	Lorence, Medina-
			10-HCPT 150	Bolivar, and Nessler,
				2004
	Callus	Shangai China	CDT 2040, 2260	Windonfold at al. 1007
	Callus	Shangai, China	10-HCPT 80-100	
	Cell cultures		CPT 2.5 –4	Sakato <i>et al.</i> , 1974:
				van Hengel <i>et al.</i> , 1992
Camptotheca lowreyana Li	Young leaves	Texas, USA	CPT 3913–5537	Li <i>et al.</i> , 2002
	Old lea <mark>v</mark> es	a plant i	CPT 909-1184	
Camptotheca	Young leaves	Texas, USA	CPT 2592-4494	Li <i>et al.</i> , 2002
yunnanensis Dode	Old leaves	1666 ALANDARD	CPT 590	
Ervatamia heyneana (Wall)	Wood and	India	CPT 1300	Gunasekera <i>et al.</i> ,
I. Cooke	Stem bark	Okinowa Janan	9-MCPT 400	1979 Airoma at al. 1088
(Wight) Sleumer	Stem wood	Okinawa, Japan	dCPT 1400-2400	Alyama <i>et al.</i> , 1988
	Stem	Taiwan	ACPT 0.24	Wu <i>et al.</i> , 1995
	Sho <mark>ot</mark>	Mahabaleshwar,	CPT 750	Roja and Heble, 1994
		India	9-MCPT 130	
	Plantlet		9-MCPT 7	
	culture			
	Callus	Cadavari India	9-MCPT 1	Stiniups and Das. 2002
	Callus	Gouavan, mula	MACPT 2.5	Ciddi and Shular, 2003
	Callus	Ooty, maia	9-MCPT traces	Cidal and Shaler, 2000
	Cell culture	Satara, India	CPT 1.1	Fulzele <i>et al.</i> , 2001
	QL ALL Q		9-MCPT 0.81	
Merriliodendron	Leaves and	Guam	CPT 530	Arisawa <i>et al.</i> , 1981
megacarpum	stem		9-MCPT 170	
(Hemsl.) Sleumer				
Mostuea brunonis Didr.	Entire plant	Lope, Gabon	CPT-20-O-b-	Dai <i>et al.</i> , 1999
			glucoside 100	
			DPMI 100 Strictoporciale 000	
			Sinclosamide 600	

 Table 2.1 Sites of accumulation of CPT and CPT derivatives in natural sources.

Species	Tissue analyzed	Sample origin	Camptothecinoids content (µg/g dry weight)	Reference
Ophiorrhiza fistipula	Leaves	-	7-MCPT	Arbain, Putra, and Sargent, 1993
<i>Ophiorrhiza kuroiwae</i> Makino	tissue cultures	Okinawa, Japan	CPT 55 10-MCPT 2	Asano <i>et al.</i> , 2009
Ophiorrhiza liukiuensis Hayata	Whole plants	Okinawa, Japan	CPT 127 9-MCPT 126 10-MCPT 30	Kitajima <i>et al.</i> , 2005
Ophiorrhiza mungos Linn.	Entire plant	Colombo, Ceylan	CPT 12 9-MCPT 10.41	Tafur <i>et al.</i> , 1976
	Shoots Roots	Kerala, India	CPT 96 9-MCPT traces CPT 176 9-MCPT traces	Roja, 2006
<i>Ophiorrhiza pumila</i> Champ.	Leaves Young roots Hairy roots	Japan	CPT 300–400 CPT 1000 CPT 1000	Saito <i>et al.</i> , 2001
	Entire plant Hairy roots	Kagoshima, Japan	CPT 300–510 9-MCPT 70–140 Chaboside 300–690 CPT 240	Yamazaki <i>et al.</i> , 2003
	Cell cultures	Japan	None	Kitajima <i>et al.</i> , 1998
Ophiorrhiza rugosa	Shoots	Kerala, India	CPT 10 9-MCPT traces	Roja, 2006
	Roots		CPT 20 9-MCPT traces	
<i>Ophiorrhiza trichocarpon</i> Blume	Whole plant	Satun, Thailand	CPT MDOCPT	Klausmeyer <i>et al.</i> , 2007
Pyrenacantha klaineana Pierre ex Exell & Mendoca	Stems	Ankasa Game Reserve, Ghana	CPT 4.8 9-MCPT 1.6	Zhou <i>et al.</i> , 2000

Table 2.1 (continued)

CPT = camptothecin; ACPT = O-acetyl-CPT; dCPT = (20S)-18,19-dehydro CPT;

10-HCPT = 10-hydroxy CPT; MACPT = 9-methoxy-20-O-acetyl-CPT; 9-MCPT = 9-methoxy CPT;

7-MCPT = 7-methoxy CPT, 10-MCPT = 10-methoxy CPT; DPMI = deoxy pumiloside;

MDOCPT = 9,10-methylenedioxy-(20S)-CPT

2.2 The genus Ophiorrhiza

2.2.1 Botanical aspects of Ophiorrhiza

Ophiorrhiza L. is a predominantly herbaceous genus and comprising about 400 species (Schanzer, 2005). The genus *Ophiorrhiza* belongs to the family Rubiaceae, the subfamily Rubioideae, the tribe Ophiorrhizeae. This genus is distributed from eastern India to the western Pacific and from southern China to northern Australia. About 44 species have been recorded from Thailand (Puff, 2007). Since the systematic knowledge of this genus is still inadequate, recent regional revisions are available only for marginal parts of its area: the Pacific, China, and the Indian subcontinent. Many herbarium collections of *Ophiorrhiza* for the coming treatment for Flora of Thailand revealed a number of specimens that could not be assigned to any of the species described so far.

The characteristics of the genus Ophiorrhiza were described in Rubiaceae of Thailand (Chamchumroon, Chayamarit and Puff, 2005) and Flora of Thailand: Rubiaceae (Puff, 2007). Ophiorrhiza L. is distinctly herbaceous plant; prostrate or erect perennial or annual, uncommonly subshrubby; stem sometimes succulent. Leaves are opposite (decussate), rarely slightly anisophyllous, and blades mostly membranous; leaf-like stipules entire or fimbriate. Inflorescence terminal often consist of helicoid or scorpioid cymes, sometimes congested and head-like; bracts well developed or absent. Flowers are 5-merous, hermaphrodite, heterostylous or isostylous, sometimes cleistogamous; calyx lobes often very small; corolla typically narrowly infundibular to hypocrateriform, tube inside glabrous or hairy, base of tube occasionally distinctly bulbous, lobes valvate in bud, ascending to reflexed in open flowers; stamen inserted at different levels in corolla tube (usually high up in short-styled, but in the low part in long-styled morphs), filaments long or short, anthers included or exerted; style filiform, with 2-lobed stigma, included or exerted (above the level of the anthers in long-styled morphs but not necessarily exserted); ovary 2-celled, each locule with numerous ovules on placenta attached to lower half of septum; roof of ovary with conspicuous 2-lobed disk. Fruits are strongly laterally compressed, capsular, obcordate, usually much broader than high, loculicidally dehiscent; seeds very numerous, small, rhomboid, vivipary sometimes observed (Tan and Rao, 1981).

2.2.2 Phylogenetic systematic of Ophiorrhiza

According to the taxonomically complex and the high morphological variability of the genus *Ophiorrhiza* (Chou *et al.*, 2006; Darwin, 1976; Kudoh *et al.*, 2001), few studies have attempted to resolve this taxonomic problem using molecular phylogenetic systematics (Nakamura *et al.*, 2006, 2007, 2010). Several DNA regions, such as ITS, *atpB-rbcL* and *trnK/matK* have been utilized to determine the species and the varieties within *Ophiorrhiza*. Four species of *Ophiorrhiza*, which comprise all species of this genus distributed in Taiwan and Japan, were examined. The molecular phylogenetic analyses conducted with these four species revealed two major clades in the trees (Figure 2.5). The genus *Hayataella* was considered to be synonymous with *Ophiorrhiza* and also included in the *Ophiorrhiza* clade.

In plant chloroplasts, the tRNA^{Lys}(UUU) gene (*trn*K) contains a intron which encodes the *mat*K open reading frame (ORF). The *trn*K intron and its encoded *mat*K ORF have generated substantial interest in the fields of plant evolution and molecular biology (Hausner *et al.*, 2006; Hilu *et al.*, 2003). Based on assessments of recoverability, sequence quality, and levels of species discrimination, *rbcL* and *mat*K genes were recommended to be used as the plant barcode to identify specimens and contribute toward the discovery of overlooked species of land plants (CBOL Plant Working Group, 2009).





2.2.3 Chemical constituents of Ophiorrhiza

CPT was isolated and identified from entire plant of *O. mungos* (Tafur *et al.*, 1976). Later, publications on isolation of the constituents from the other *Ophiorrhiza* spp. were reported including *O. fistipula*, *O. kuroiwae*, *O. liukiuensis*, *O. pumila*, *O. rugosa*, and *O. trichocarpon* (Table 2.1). CPT, its derivatives, and CPT-related alkaloids isolated from these plants are summarized in Table 2.2.

At the cellular level, the previous study of the distribution of CPT in different tissues of *O. pumila* suggested that the highest levels of CPT accumulation were found in flower buds, young leaves, and roots (Yamazaki *et al.*, 2003). At the subcellular level, the study of hairy roots of *O. pumila* indicated that CPT is biosynthesized at the endoplasmic reticulum and transported to accumulate in a vacuole *via* vesicles (Sirikantaramas *et al.*, 2007) (Figure 2.6). It has been proposed that the lipophilic form of alkaloids is protonated to the hydrophilic form in the acidic conditions of the vacuole (Matile, 1976). As a result, the protonated form cannot move across a tonoplast membrane. After CPT is formed, it is partly stored in the vacuole, a site for avoiding self-toxicity. However, some part of the compound can diffuse freely inside the cytoplasm before excretion due to its lipophilicity (Figure 2.6). The cytoplasmic CPT might be expected to interfere with Topl in the nucleus.

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Chemical compound	Chemical structure	Reference
Camptothecin		Asano <i>et al.</i> , 2009 Kitajima <i>et al.</i> , 2005 Klausmeyer <i>et al.</i> , 2007 Roja, 2006 Saito <i>et al.</i> , 2001 Tafur <i>et al.</i> , 1976 Yamazaki <i>et al.</i> , 2003 Zhou <i>et al.</i> , 2000
7-Methoxy CPT	OCH3 CH3 CH3 CH3 CH3 CH3 CH3 CH3 CH3 CH3	Arbain <i>et al.</i> , 1993
9-Methoxy CPT	OCH3	Kitajima <i>et al.</i> , 2005 Roja, 2006 Tafur <i>et al.</i> , 1976 Yamazaki <i>et al.</i> , 2003 Zhou <i>et al.</i> , 2000
10-Methoxy CPT	H ₃ CO	Asano <i>et al.</i> , 2009 Kitajima <i>et al.</i> , 2005
10-Hydroxy CPT		Yamazaki <i>et al.</i> , 2003

 Table 2.2 Chemical constituents and structures found in Ophiorrhiza spp.

Table 2.2 (continued)

Chemical compound	Chemical compound Chemical structure		
9,10-Methylenedioxy CPT		Klausmeyer <i>et al.</i> , 2007	
Chaboside	Glc-D-β-O 19 N N N OH OH	Yamazaki <i>et al</i> ., 2003	
Lyalosidic acid	H COOH H GGlc	Kitajima <i>et al.</i> , 2005	
Mappicine	C C N C N C OH	Yamazaki <i>et al.</i> , 2003	

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Table 2.2 (continued)

Chemical compound	Chemical structure	Reference
Pumiloside		Kitajima <i>et al.</i> , 2005 Yamazaki <i>et al.</i> , 2003
Deoxy pumiloside		Kitajima <i>et al.</i> , 2005 Yamazaki <i>et al.</i> , 2003
Strictosamide		Kitajima <i>et al.</i> , 2005
Strictosidinic acid	NH H H ^W H OGlc	Yamazaki <i>et al.</i> , 2003

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Figure 2.6 Proposed models for CPT transport, accumulation, and excretion in the hairy root cell of *O. pumila*. Dots represent CPT molecules. Black arrows indicate CPT trafficking pathways. Dots in circles represent vesicle-mediated CPT transport. Dashed arrow indicates possible outward transport from the vacuole (source: Sirikantaramas *et al.*, 2007).

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2.2.4 Ophiorrhiza distributed in Thailand

Ophiorrhiza existing in Thailand has been recorded for 44 species (Puff, 2007). Only some species have local name in Thai (Smitinand, 2001; Puff and Chayamarit, 2006). Many species of *Ophiorrhiza* were recorded as the endemic and rare plants of Thailand (Santisuk *et al.*, 2006). Since Flora of Thailand (Rubiaceae) is on a process of revision, many species are still not fully resolved (Chamchumroon and Puff, 2003). The *Ophiorrhiza* spp. distributed in Thailand were described in Table 2.3.



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Botanical name	Local name	Distribution	Reference
<i>O. alata</i> Craib	ผักหลอดดอกขาว	South eastern: Chantaburi	Smitinand, 2001
<i>O. ankae</i> Craib	สร้อยกะจับ	Northern: Chiang Mai,	Smitinand, 2001
		Nan, Tak	Chamchumroon <i>et al.</i> , 2005
O. brachycarpa	-	Koh Chang, Trat	Chamchumroon and Puff, 2003
O. communis Ridl.	เป็นเบรัคนาสิ	Malay-Yala	Smitinand, 2001
			Chamchumroon <i>et al.</i> , 2005
O. harrisiana	-	Koh Chang, Trat	Chamchumroon and Puff, 2003
<i>O. hispidula</i> Wall. ex G.Don	หญ้าตื่นมือตุ๊ดตู่	Surat Thani	Smitinand, 2001
<i>O. hispidula</i> Wall. ex G.Don	-	Doi Chiang Dao, Chiang	Putiyanan and Maxwell, 2007
var. hispidula		Mai	
<i>O. kratensis</i> Craib	กะเสิมหิน	Trat	Smitinand, 2001
O. larseniorum Schanzer	-/// 3	Peninsular: Surat Thani	Schanzer, 2005
O. longifloriformis Schanzer	- / / 5.6	Koh Chang, Trat	Schanzer, 2005
O. pedunculata Schanzer	- / /))	Northern: Mae Hong Son,	Schanzer, 2004
(<i>O. hispidula</i> B. Heyne ex	1000	Chiang Mai, Chiang Rai;	
Hook. f. var.	10000	South western: Thong Pha	
longipedunculata Craib)		Phum, Kanchanaburi	
O. pseudofasciculata	7.	Northern: Chang Mai, Nan,	Chamchumroon <i>et al.</i> , 2005
Schanzer		Chiang Rai, Lampang	Schanzer, 2005
<i>O. ripicola</i> Craib	แดงก่อนจาก	Doi Inthanon National	Smitinand, 2001
ର୍	เย้าโทย	Park, Chiang Mai	Chamchumroon <i>et al.</i> , 2005
		N a N D III	Puff and Chayamarit, 2007
O. rugosa	-	Koh Chang, Trat	Chamchumroon and Puff, 2003
O. schmidtiana Craib	ผักพรหมมิ	South eastern	Smitinand, 2001
O. trichocarpon Blume	ผักสามชาย	Khao Yai National Park,	Puff and Chayamarit, 2006
		Northern: Chiang Mai,	Chamchumroon <i>et al.</i> , 2005
		Chiang Rai, Phayao	Schanzer, 2004
O. trichocarpon Blume	-	South eastern: Sa Kaeo	Schanzer, 2004
var. <i>glabra</i> Schanzer			
<i>O. villosa</i> Roxb	-	Western, Northern: Doi	Putiyanan and Maxwell, 2007
		Chiang Dao, Chiang Mai	Schanzer, 2004

 Table 2.3 Ophiorrhiza spp. distributed in Thailand.
2.3 Topoisomerase I enzyme (TopI)

2.3.1 Cellular role and structure of Topl

DNA topoisomerases are nuclear enzymes that make transient strand breaks in DNA to allow a cell to manipulate its topology (Osheroff, 1998). Every cell type so far examined contains DNA topoisomerases for cell growth. During DNA replication, the two strands of the DNA must become completely unlinked by topoisomerases, and during transcription, the translocating RNA polymerase generates supercoiling tension in the DNA that must be relaxed (Wang, 1996). There are two classes of topoisomerase, known as type I and type II enzyme. Those enzymes that cleave only one strand of the DNA are defined as type I (Champoux, 2001). In contrast, type II enzyme make a transient double-stranded break in DNA and pass a separate double-stranded molecule through the break.

Eukaryotic type I topoisomerase (topoisomerase I, TopI) is classified as type IB subfamily members (formerly called typeI-3'), the TopI that cleaves the DNA by becoming covalently linked to the 3' DNA terminus (Pommier *et al.*, 1998). The 91-kDa human TopI protein has been subdivided into four distinct domains (Figure 2.7). The N-terminal domain contains putative signals for the enzyme's nuclear localization. The core domain is essential for the relaxation of supercoiled DNA; it shows a high phylogenetic conservation, particularly in the residues closely interacting with the double helix. The C-terminal domain contains the active site enzyme tyrosine, which forms a transiently covalent phosphodiester bond between TopI and the DNA (González *et al.*, 2007). Strand scission occurs through a transesterification in which a tyrosine hydroxyl group of TopI is covalently linked to the 3' phosphate of a phosphodiester bond, liberating the 5' hydroxyl to generate a strand break (Champoux, 1981) (Figure 2.8). DNA religation occurs in the following step to release TopI from the cleavage complexes and to repair TopI-mediated DNA damage.

	N-terminal domain	Core domain	Linker C-terminal domain domain
N-			– C
1	215		636 713 765
	Poorly conserved,	Highly conserved,	Poorly Conserved,
	highly charged,	binds DNA,	conserved. contains
	unstructured,	contains most catalytic residues.	active site
	protease sensitive,		Tyr723.
con	ntains targeting signals.		

Figure 2.7 Domain structure of human Topl. Human Topl comprises an N-terminal domain (open box), a core domain (gray box), a linker domain (diagonally striped box), and a C-terminal domain (black box). The domain boundaries are based on sequence alignments, limited proteolysis studies, and the crystal structures of the protein (source: Champoux, 2001).

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Figure 2.8 Human Topl-mediated DNA cleavage and religation. Y723 refers to the tyrosine involved in the transesterification reaction with the DNA. By convention, the bases flanking the top1 cleavage site are referred to as -1 and +1 for the bases at the 3' and 5' DNA termini, respectively (Pommier *et al.*, 1998).

2.3.2 Mechanism of Topl-targeted CPT

CPT and its derivatives exhibit antitumor activity due to their interacting with the cellular Topl (Hsiang *et al.*, 1985). This interaction damages the DNA, causing the cancer cell to be destroyed or preventing the cancer cell from growing and reproducing (Pommier, 1998). CPT are named topoisomerase "poisons" to distinguish them from conventional enzyme inhibitors. CPT does not bind to Topl alone but it stabilizes a covalent complex between Topl and the nicked DNA (Reid, Benedetti, and Bjornsti, 1998). Trapping of the cleavable complex and preventing DNA re-ligation could be poison into the cells (Figure 2.9). The collision of advancing replication forks with compound-stabilized intermediates appears to produce the cytotoxic DNA lesions that signal cell cycle arrest and cause cell death (Strumberg *et al.*, 2000). Therefore, inhibitory of Topl activity was a great harm to a cellular genome to develop a nuclear toxin that can efficiently kill cancer cells.

CPT and its derivatives have been studied as potent inhibitors of replication, transcription, and packing of double stranded DNA-containing adenoviruses, papovaviruses, and herpesviruses, and the single-stranded DNA-containing autonomous parvoviruses (Pantazis *et al.*, 1999). CPT was also shown to have promising activity against parasitic trypanosomes and Leishmania (Bodley and Shapiro, 1995). CPT, 9-MCPT, 10-MCPT and 9,10-methylenedioxy CPT showed functional inhibition of the hypoxia-inducible factor 1 α (HIF-1 α), a master regulator of the cancer cell's ability to survive under oxygen deprivation (Klausmeyer *et al.*, 2007). Hence, these drugs may have other desirable activities against solid tumors that are independent of Topl poisoning.



Figure 2.9 Mechanism of action of CPT. Relevant events in determining the cytotoxic potency of CPT and its derivatives are: (a) uptake, (b) lysosomal or mitochondrial sequestration and, (c) nuclear localization and stabilization of the "cleavable complex" (source: Lorence and Nessler 2004).

The selective cytotoxicity of CPT for tumor cells depends on the level of Topl activity and the rate of repair of the replication induced double-strand break (Gupta, Fujimori, and Pommier, 1995). Cell lines which have high levels of Topl enzyme are hypersensitive to CPT-induced cytotoxicity, such as colon adenocarcinoma, ovarian and esophageal carcinoma. Although Topl is expressed throughout the cell cycle, cells in S-phase are 1000 times more sensitive than cells in G₁ or G₂- phase reflecting the need for DNA replication for drug efficacy (Del Bino, Lassota, and Darzynkiewic, 1991).

2.3.3 Topl of CPT-resistant cancer cell

CPT analogs have proven to be effective anticancer drugs; however, resistance is still a critical clinical problem (Rasheed and Rubin, 2003). There are several different ways by which resistance to CPT could hamper the treatment in cancer patients, such as reduced drug uptake, overexpression of P-glycoprotein, and mutation in *Top*I gene which results in altered TopI structure or function leading to decrease in enzyme activity and ability of CPT to stabilize the cleavable complex (Gupta *et al.*, 1995). Previous study found that normal cell can express both wild-type and mutant TopI, whereas CPTresistant cancer cells express mutant TopI only (Wang *et al.*, 1997). Besides, the cancer cells expressing mutant TopI contain similar level and activity of TopI, compared with the normal cell.

Most of the mutations are contained in well-conserved regions of the Topl, the core and the C-terminal domains, which are critical for catalytic activity and interaction with CPT (Gupta et al., 1995). The three-dimensional structure of human Topl suggests four regions that can be mutated to produce a CPT-resistant Topl (Redinbo et al., 1998) (Table 2.4). These residues may play a structural role in the proper packing of the Cterminal and core domains and may affect CPT efficacy by interfering with the positioning of catalytic or CPT-binding residues. Other residues and substitutions in human Topl which confer resistance in CPT-resistant cancer cells were summarized in Table 2.4. Some of these point mutations in human Topl were also found at the corresponding position in the CPT-resistant yeast and vaccinia viruses. For example, N726S and N726D substitutions in CPT-resistant yeast Topl are at the corresponding 722 position of human Topl (Fertala et al., 2000).

No.	Residues and	Deference
	substitution	Releience
1	F361 to M370 region	Redinbo <i>et al.</i> , 1998
2	F361S	Chrencik <i>et al.</i> , 2004
		Rubin <i>et al.</i> , 1994
3	G363C	Benedetti <i>et al.</i> , 1993
4	R364H	Urasaki <i>et al.</i> , 2001
5	M370T	Gupta <i>et al.</i> , 1995
6	G503S	Pommier <i>et al.</i> , 1998
7	K532 to S534 region	Redinbo <i>et al.</i> , 1998
8	D533N	Rasheed and Rubin, 2003
	D533G	Tamura <i>et al.</i> , 1990
9	D583G	Pommier <i>et al</i> ., 1998
10	G717 to N722 region	Redinbo <i>et al.</i> , 1998
11	G717V	Wang et al., 1997
12	L721R	Gupta <i>et al.</i> , 1995
13	N722S	Chrencik et al., 2004
	N722S, N722A	Gupta <i>et al.</i> , 1995
14	Y723F	Woo <i>et al.</i> , 2002
15	1725R	Rasheed and Rubin, 2003
16	N726S/A	Woo <i>et al.</i> , 2002
9	Y727F	Woo <i>et al.</i> , 2002
17	T729A	Kubota <i>et al.</i> , 1992
	T729	Redinbo <i>et al.</i> , 1998
	T729I	Wang <i>et al.</i> , 1997
18	G737S	Rasheed and Rubin, 2003

 Table 2.4 Point mutations at residues of CPT-resistant human Topl.

2.3.4 Topl of CPT-producing plants

Many CPT-producing plants, including *C. acuminata*, *O. pumila* and *O. liukiuensis*, have Topl enzymes with several point-mutations that confer resistance to CPT (Sirikantaramas *et al.*, 2008). Three amino acid substitutions that contribute to CPT resistance were identified: N421K, L530I, and N722S (numbered according to human Topl). The proposed amino acids that are involved in catalytic function or affect CPT binding are shown in Figure 2.10. Asp-533 and Ser-722 directly bind to CPT. Other residues that indirectly bind to CPT are important for the proper positioning of Asp-533 and Ser-722.

The crystal structure of human Topl in a covalent complex with duplex DNA containing topotecan suggests that mutations of these amino acids would disrupt drug binding (Sirikantaramas *et al.*, 2008). These mutations suggest the effect of an endogenous toxic metabolite on the evolution of the target cellular component. A phylogenetic tree based on *Topl* sequences of CPT-producing and non-CPT-producing organisms revealed a close relationship of CPT-producing plants, *O. pumila* and *O. liukiuensis*, and a separation of *O. japonica*, a non-CPT-producing plant (Figure 2.11).

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									Resid	lue nu	mber								
	532	590	632	723	361	363	364	367	418	420	421	502	503	530	533	619	722	725	729
	Ca	talytic	funct	ion						Direc	t/Ind	irect C	PT bir	nding					
Hs	К	R	Н	Υ	F	G	R	Н	Е	1	Q	V	G	L	D	Y	Ν	D	Т
At	К	R	Н	Y	F	G	R	Н	D	I	Ν	V	G	L	D	Y	Ν	D	Т
Cr	К	R	Н	Y	F	G	R	Н	D	I	Ν	V	G	L	D	Y	Ν	D	Т
Oj	К	R	Н	Y	F	G	R	Н	D	I	Ν	V	G	L	D	Y	Ν	D	Т
Ор	К	R	Н	Y	F	G	R	Н	D	V	Ν	V	G		D	Y		D	Т
01	К	R	Н	Y	F	G	R	Н	D	1	N	V	G		D	Y		D	Т
Ca	К	R	Н	Y	F	G	R	Н	D	1	К	V	G	L	D	Y	S	D	Т

Figure 2.10 Amino acid polymorphism in Topl of CPT-producing plants and nonproducing organisms. The numbering is based on human Topl. The red characters indicate the amino acid substitutions in Topl of CPT-producing plants. *Hs*, *Homo sapiens*; *At*, *Arabidopsis thaliana*; *Cr*, *Catharanthus roseus*; *Oj*, *Ophiorrhiza japonica*; *Op*, *Ophiorrhiza pumila*; *Ol*, *Ophiorrhiza liukiuensis*; *Ca*, *Camptotheca acuminata* (source: Sirikantaramas *et al.*, 2008).





Figure 2.11 Neighbor-joining tree of *TopI* sequences of plants, yeast, and humans. The numbers indicate bootstrap values. CPT production is indicated by "+" or "-" (source: Sirikantaramas *et al.*, 2008).

2.4 Definition and previous studies of coevolution

The theory of coevolution was briefly described in the Origin of Species (Darwin, 1859) that populations evolve over the course of generations through a process of natural selection, and presented a body of evidence that the diversity of life arose through a branching pattern of evolution and common descent. Currently, the concept of coevolution (covariation/correlated mutation) is the change of a biological object triggered by the change of a related object (Yip *et al.*, 2008). Coevolution can occur at multiple levels of biology: it can be as microscopic as covarying traits between different species in an environment.

Species-level coevolution includes the evolution of a host species and its parasites, for instance, the coevolution between the resistance gene and the virulence gene of host plants and their fungal pathogens (Frank, 1993). The interdependent plant-vertebrate seed dispersal systems suggest the coevolution of plant-animal (Herrera, 1985). Genetic coevolution includes the coding genes of some interacting proteins are preserved or eliminated together in new species (Pellegrini *et al.*, 1999), or have similar phylogenetic trees (Goh *et al.*, 2000). At the amino acid level, some residues under physical or functional constraints exhibit correlated mutations (Gloor *et al.*, 2005; Socolich *et al.*, 2005; Süel *et al.*, 2003). A corresponding mutation in two position of the multiple sequence alignment may propose residues which are functionally or structurally important, or possibly key sites of interaction between the protein and its substrate (Martin *et al.*, 2008).

The mutation in Topl of CPT-producing plants suggests the coevolution between CPT biosynthetic pathway and self-resistance mechanism (Sirikantaramas, Yamazaki, and Saito, 2009). The *Ophiorrhiza* genus is composed of both CPT-producing and non-producing species. This provides a great benefit to follow the coevolution of the CPT biosynthetic pathway and Topl mutation as a self-resistance mechanism.

CHAPTER III

THE PRODUCTION OF CAMPTOTHECIN

In this study, CPT productions by Thai *Ophiorrhiza* plants were explored for the first time. Plant specimens were collected from various locations of Thailand in order to find numerous species for coevolution study and for novel alternative sources of CPT. The methanol extracts of plant samples were analyzed using HPLC/DAD/ESI/MS. Standard solutions of CPT, CPT derivatives, and chemical compounds involved in CPT biosynthesis pathway were also analyzed using the same method.

3.1 Materials and Methods

3.1.1 Plant specimen collection

Ophiorrhiza species distributed in Thailand were collected from previously reported provinces (Chamchumroon and Puff, 2003; Schanzer, 2004, 2005), including Trat, Kanchanaburi, Chiang Mai, Chaing Rai and Lampang. Phuket, Chantaburi and Nakorn Ratchasima were also investigated for *Ophiorrhiza* plants. Numbers of specimens collected from each locality depended on their abundance in the native habitats and their different features. The collected plants were then cultivated in the Medicinal Plant Garden of the Faculty of Pharmaceutical Sciences, Chulalongkorn University. All specimens were identified to species-level by Ivan A. Schanzer, Ph.D. from Herbarium Main Botanical Garden, Russia (Table 3.1). The specimens were deposited at the Museum of Natural Medicines, Faculty of Pharmaceutical Garden Herbarium, Chiang Mai Province, Thailand. Some of fresh *Ophiorrhiza* specimens are shown in appendix A.

Species	Specimen	Vouchar No	Locality (area, province)	Part of	
Species	No.	voucher no.	Locality (alea, province)	Thailand	
<i>O. fucosa</i> Hance	ophi 32-33	BH-090519-032,	Khao Soi Dao Nua, Chantaburi	SE	
		033			
	ophi 34	VV-090523-034	Khao Soi Dao Tai, Chantaburi	SE	
	ophi 64	VV-090924-064	Phlio National Park, Chantaburi	SE	
	ophi 65	VV-090924-065	Khlong Narai waterfall,	SE	
			Chantaburi		
<i>O. harrisiana</i> B. Heyne ex	ophi 14-27	VV-090502-014-	Than Mayom waterfall,	SE	
Hook. f.		027	Ko Chang, Trat		
<i>O. pedunculata</i> Schanzer 🥌	ophi 28-31	VV-090925-028-	Erawan National Park,	SW	
(O. hispidula Wall. ex G.Don		031	Kanchanaburi		
var. longipedunculata Craib)	ophi 41-42	VV-090708-041	Mok Fa waterfall, Chiang Mai	N	
	ophi 47	VV-090806-047	Tard Mok waterfall, Chiang Mai	N	
	ophi 57	ISC-090919-057	Chae Son National Park,	N	
		21212121	Lampang		
	op <mark>h</mark> i 60	ISC-090919-060	Mae Yom National Park,	N	
	(See)	2112/11/200	Lampang		
0	ophi 66-67	VV-090925-066-	Erawan National Park,	SW	
		067	Kanchanaburi		
<i>O. plumbea</i> Craib	ophi 1-13	VV-090421-001-	Bangpae waterfall, Phuket	WS	
	10	013			
O. pseudofasciculata	ophi 37	BH-090726-037	Doi Suthep-Pui National Park,	N	
Schanzer			Chiang Mai		
ລາກາລ	ophi 54	ISC-090920-054	Chae Son National Park,	N	
า พุพาศา	11199	99119	Lampang		
	ophi 62	ISC-090918-062	Khun Kon waterfall, Chiang Rai	N	
	ophi 63	ISC-090917-063	Doi Pha Hom Pok National	N	
			Park, Chiang Mai		

 Table 3.1 Eight Ophiorrhiza species in this study, their voucher numbers, and localities.

Table 3.1 (continued)

Creation	Specimen			Part of	
Species	No.	voucher No.	Locality (area, province)	Thailand	
O. ridleyana Craib	ophi 52-53	VV-090806-	Queen Sirikit Botanical	N	
		052, 053	Garden, Chiang Mai		
	ophi 55-56	ISC-090919-	Chae Son National Park,	Ν	
		055, 056	Lampang		
	ophi 61	ISC-090913-	Mae Yom National Park,	N	
		061	Lampang		
O. trichocarpon Blume	ophi 44-45	VV-090 <mark>806-</mark>	Mok Fa waterfall, Chiang Mai	N	
var. glabra Schanzer		044-045			
	ophi 46	VV-090806-	Tard Mok waterfall, Chiang Mai	N	
		046			
	ophi 51	VV-090808-	Queen Sirikit Botanical	N	
		051	Garden, Chiang Mai		
/	ophi 58	ISC-090919-	Chae Son National Park,	N	
		58	Lampang		
	ophi 68	VV-090926-	Khao Yai National Park,	NE	
	Ser.	068	Nakorn Ratchasima		
Ophiorrhiza sp. 35	ophi 35-36	VV-090523-	Rambhai Barni Rajabhat	SE	
		035, 036	University, Chantaburi		

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3.1.2 Methanol extraction

According to the previous study of alkaloid accumulation in *O. pumila* (Yamazaki *et al.*, 2003), young leaves and roots which contained high amount of CPT were used as materials for CPT analysis in this study. Specimens from different localities of each species were examined for CPT analysis. Young leaves and roots of *Ophiorrhiza* samples were freeze-dried and ground using liquid nitrogen with Multi-beads shocker[®] (Yasui kikai Co, Japan) at 1500 rpm for 10 s and stored in a vacuum desiccator overnight. The dried specimens were weighed and extracted with MeOH 10 mg/1 mL (dry weight), following by ultrasonication for 30 min and kept at 4°C overnight. The crude extracts were centrifuged at 15000 g for 10 min. The supernatants were filtered through 0.45-µm filters (Millipore Co, USA) and analyzed by Agilent 1100 series HPLC/DAD/ESI/MS (Palo Alto, CA, USA).

3.1.3 HPLC-MS analysis

HPLC analyses were carried out using a Mightysil RP-18 column (5 mm, 250 mm × 4.6 mm, Kanto Chemical Co Inc, Japan) at a flow rate of 0.8 mL/min. Elution gradient was as follows: 0–35 min linear gradient from solvent A $[H_2O:HOAc:MeOH (79.8:0.2:20)]$ to solvent B $[H_2O:HOAc:MeOH (9.975:0.025:90)]$, 35–40 min isocratic at 100% of solvent B. Each examined sample was analyzed three times. All samples in each time analysis were randomly injected into a system. The HPLC-MS system was set to 4°C. The standard compounds in powder form were dissolved in MeOH to prepare standard solution (Appendix B). Standard solutions of CPT, 9-methoxy camptothecin (9-MCPT), 10-hydroxy camptothecin (10-HCPT), pumiloside (PMI), deoxy pumiloside (DPMI), chaboside and mappicine were analyzed using the same method (Yamazaki *et al.*, 2003). CPT and other compounds in *Ophiorrhiza* samples were identified by their MS spectra, UV spectra at 254-nm detection and retention times compared with those of standard compounds. The contents of compounds detected in each sample were

calculated. Average contents and standard deviation (SD) of triplicate analyses were calculated.

3.2 Results

3.2.1 Species identification

Ophiorrhiza specimens collected in this study were identified into eight species (Table 3.1). Only one sample: *Ophiorrhiza* sp. 35, from Rambhai Barni Rajabhat University, Chantaburi Province, could not be determined a specific species. According to the morphological identification, Ophi 62 was not assured being *O. pseudofasciculata* (Appendix A, Figure A7).

3.2.2 HPLC-MS results

HPLC-DAD chromatograms monitored at 254 nm, UV spectra and mass spectra of standard compounds were shown in Appendix B. CPT, 9-MCPT, 10-HCPT, PMI, and DPMI were detected in the samples (Table 3.2). Chaboside and mappicine were not found in any samples. The average contents of detected compounds of five species, *O. fucosa*, *O. harrisiana*, *O. plumbea*, *O. ridleyana* and *Ophiorrhiza* sp. 35, were calculated (Figure 3.1, 3.2, 3.4–3.6).

HPLC-DAD chromatograms of some samples, including Ophi 64 (*O. fucosa*), revealed a peak at a retention time approximately 26.4 min eluted earlier than 9-MCPT peak at 26.9 min (Figure 3.8). This compound had UV and mass spectrum patterns similar to those of 9-MCPT. Thus, it was named 9-MCPT analog and was calculated for the content by comparing with 9-MCPT standard. The average contents of 9-MCPT analog in five *Ophiorrhiza* spp. were shown in Figure 3.3.

From eight species of collected *Ophiorrhiza*, CPT was found in the root extracts of four species: *O. fucosa*, *O. harrisiana*, *O. ridleyana* and *O. plumbea* and in the leaf

extract of one species, *O. harrisiana.* 9-MCPT and 9-MCPT analog were detected in CPT-detected species and also in the leaf extract of *Ophiorrhiza* sp. 35. 10-HCPT was detected only in the root and leaf extracts of Ophi 56 (*O. ridleyana*). PMI and DPMI were detected in four CPT-detected species. Average contents of CPT and CPT derivatives in each species were calculated (Figure 3.7). The highest amounts of CPT and 9-MCPT analog were detected in the leaf extracts of *O. harrisiana*. The highest amounts of 9-MCPT was detected in the root extract of *O. harrisiana*.



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Species	Locality (area, province)	Specimen	Voucher No.	С	PT	9-M	CPT	9-M ana	CPT alog	10-H	ICPT	Р	MI	DF	PMI
		No.	1	L	R	L	R	L	R	L	R	L	R	L	R
O. fucosa	Khao Soi Dao Nua, Chantaburi	Ophi 32	BH-090519-032	-	+	-	-	-	+	-	-		+	-	_
	Khao Soi Dao Tai, Chantaburi	Ophi 34	VV-090523-034	-	+	-	+	+	+	-	-	-	+	-	+
	Phlio National Park, Chantaburi	Ophi 64	VV-090924-064	-	+	-	+	-	+	-	-	-	+	-	+
	Khlong Narai waterfall, Chantaburi	Ophi 65	VV-090924-065	-	+	-	+	-	+	-	-	-	+	-	+
O. harrisiana	Than Mayom waterfall, Ko Chang, Trat	Ophi 18	VV-090502-018	+	+	+	+	+	+	-	_	_	+	-	+
		Ophi 25	VV-090502-025	+	+	+	+	+	+	_	_	-	+	-	+
		Ophi 26	VV-090502-026	+	+	-	-	+	+	-	-	+	+	-	-
		Ophi 27	VV-090502-027	+	+	+	-	+	+	-	-	+	+	_	-
O. pedunculata	Erawan National Park, Kanchanaburi	Ophi 31	VV-090925-031	-	-	-	-	-	-	-	-	-	-	-	-
		Ophi 67	VV-090925-067	-	-	-	-	-	-	-	-	-	-	-	-
	Mork Fa waterfall, Chiang Mai	Ophi 41	VV-090708-041	-	-	-	-	-	-	-	-		-	_	-
	Jae Son National Park, Lampang	Ophi 57	ISC-090919-057	-	-	-	-	-	-	-	-	-	-	-	-
O. plumbea	Bangpae waterfall, Phuket	Ophi 3	VV-090421-003	-	+	-	+	+	+	-	-	-	+	-	+
		Ophi 4	VV-090421-004	-	+	-	+	-	+	-	-	-	-	-	-
		Ophi 6	VV-090421-006	-	+	-	+	-	+	-	-	-	+	-	+
O. pseudofasciculata	Doi Suthep-Pui National Park, Chiang Mai	Ophi 37	BH-090726-037	-		-	-	-	-	-	-	-	-	-	-
	Jae Son National Park, Lampang	Ophi 54	ISC-090920-054	-	~ 2	-	-	-	-	-	-	-	-	-	-
	Khun Kon waterfall, Chiang Rai	Ophi 62	ISC-090918-062	-	-	-	-	-	-	-	-	-	-	-	-
	Doi Pha Hom Pok National Park, Chiang Mai	Ophi 63	ISC-090917-063	-	-	-	-	-	-	-	-	-	-	-	-
O. ridleyana	Queen Sirikit Botanical Garden, Chiang Mai	Ophi 52	VV-090806-052	-	-	-	-	-	-	-	-	-	-	-	-
	Jae Son National Park, Lampang	Ophi 55	ISC-090919-055	-	+	+	+	-	+	-	-	-	+	-	+
	ର ୩ ୧	Ophi 56	ISC-090919-056	-	+	+	+	-	+	+	+	-	+	-	+
	Mae Yom National Park, Lampang	Ophi 61	ISC-090913-061	J -	+	+	+	-	-	-	-	-	+	-	+
O. trichocarpon	Queen Sirikit Botanical Garden, Chiang Mai	Ophi 51	VV-090808-051	-	-	-	-	-	-	-	-	-	-	_	-
var. glabra	Jae Son National Park, Lampang	Ophi 58	ISC-090919-058	-	-	-	2	-	-	-	-	-	-	-	-
	Khao Yai National Park, Nakorn Ratchasima	Ophi 68	VV-090926-068		-	- /	_	-	-	-	-	_	-		_
Ophiorrhiza sp. 35	Rambhai Barni Rajabhat University, Chantaburi	Ophi 35	VV-090523-035	-		+	+) -	-	_	-	-	-	-	-
	9	Ophi 36	VV-090523-036	-	-	+	+	-	-	-	-	-	-	-	-

Table 3.2 HPLC-MS results of compounds detected in the leaf (L) and root (R) extracts of eight Ophiorrhiza spp.

'+' detected

'-- ' non-detected



Figure 3.1 Camptothecin content (% dry weight) in the leaf and root extracts of each *Ophiorrhiza* samples. Each bar represents the mean ± SD of triplicate analyses.



Figure 3.2 9-methoxy camptothecin content (% dry weight) in the leaf and root extracts of each *Ophiorrhiza* samples. Each bar represents the mean ± SD of triplicate analyses.



Figure 3.3 9-methoxy camptothecin analog content (% dry weight) in the leaf and root extracts of each *Ophiorrhiza* samples. Each bar represents the mean ± SD of triplicate analyses.





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Figure 3.5 Pumiloside content (% dry weight) in the leaf and root extracts of each *Ophiorrhiza* samples. Each bar represents the mean ± SD of triplicate analyses.



Figure 3.6 Deoxy pumiloside content (% dry weight) in the leaf and root extracts of each *Ophiorrhiza* samples. Each bar represents the mean ± SD of triplicate analyses.



Figure 3.7 Average contents (% dry weight) of CPT and its derivatives in the leaf (L) and root (R) extracts of each *Ophiorrhiza* species.



Figure 3.8 HPLC-DAD chromatogram of the root extract of Ophi 64 showing a peak of unknown compound, 9-MCPT analog, at a retention time 26.4 min and a peak of 9-MCPT at 26.9 min.

3.3 Discussion

The distribution of Ophiorrhiza spp. collected in this study (Table 3.1, Fig 3.9) showed some interesting viewpoints. Ophiorrhiza in northern part of Thailand had high species diversity. For instance, there were four species collected in one location, Chae Son National Park, Lampang that were O. pedunculata, O. pseudofasciculata, O. ridleyana, and O. trichocarpon. Even Mok Fa and Tard Mok waterfall in Chaing Mai Province are small areas, two species were found. Ophiorrhiza in other parts of Thailand had lower diversity comparing with the northern part. For instance, Chantaburi Province in south-eastern part was found two species in four locations. We can imply that the northern areas are appropriate for the growth of Ophiorrhiza plants. According to previous study (Schanzer, 2004) and our collecting experience, most Ophiorrhiza habitats were along streams and waterfalls on humus, open soil, wet rocks, in evergreen, mixed, or disturbed bamboo dominated forests. They required humid climate with shade, not directed sunlight. For intraspecies aspect, O. pedunculata and O. trichocarpon collected in this study had wide distribution comparing with O. pseudofasciculata and O. ridleyana which were found only in northern part of Thailand. O. fucosa which has never been reported in Thailand were found only in Chantaburi Province.

Although there was a fluctuation of CPT content, the presence or absence, and part of accumulation of CPT detected in samples within species, but Ophi 52, were congruent (Figure 3.1), despite their various localities of collection (Table 3.1). Conversely, different *Ophiorrhiza* spp. grown naturally in the same area had different CPT-producing abilities (e.g. *O. pseudofasciculata* and *O. ridleyana* from Chae Son National Park, Lampang). From these results, we propose that *Ophiorrhiza* in Thailand had CPT production abilities which were mainly related to species, not habitat. Based on our hypothesis, Ophi 52 should not be *O. ridleyana*; only its morphological characteristics looked similar to this species. Thus, we expected that *mat*K and *Top*I sequence analysis results can resolve this problem.



Figure 3.9 Distribution of *Ophiorrhiza* spp. collected in this study: A. *O. fucosa*; B. *O. harrisiana*; C. *O. pedunculata*; D. *O. plumbea*; E. *O. pseudofasciculata*; F. *O. ridleyana*; G. *O. trichocarpon*; H. *Ophiorrhiza* sp. 35. Black shapes represent CPTand CPT derivatives-producing *Ophiorrhiza* spp. White-centered shapes represent non-CPT- producing *Ophiorrhiza* spp.

Unlike CPT, some compounds had incongruent detection results in samples within species. For instance, 9-MCPT was not detected in Ophi 32, among other *O. fucosa* plants and 9-MCPT analog was not detected in Ophi 61, among other *O. ridleyana*. However, it was noticeable that most samples showed relative contents of detected compounds within species (Figure 3.1-3.6). For instance, among *O. harrisiana*, Ophi 18 had the highest content of CPT, 9-MCPT, 9-MCPT analog, including PMI and DPMI. Among *O. fucosa*, Ophi 32 had the lowest content of CPT, 9-MCPT analog, and PMI, whereas 9-MCPT and DPMI were not detected. In fact, PMI and DPMI are the indicators of CPT production. This study, PMI and DPMI were detected in the CPT accumulating parts in CPT-producing species. From these results, we can imply that the plants which had relative contents of PMI, DPMI, and CPT might be in a CPT-production phase. From the relation of CPT and CPT derivative contents, we still cannot conclude that CPT derivatives are produced earlier of the CPT production.

From HPLC-MS results (Table 3.2), 9-MCPT analog was detected in all 9-MCPTcontaining species. 9-MCPT analog were eluted earlier than 9-MCPT for 0.5 min approximately (26.4 min of 9-MCPT analog and 26.9 min of 9-MCPT). Mass spectra of these two compounds showed the major ion *m*/*z* 379.0 [M + H]⁺ (Appendix B). A recent study (Shweta *et al.*, 2010) reported an identification of 9-MCPT isomer, 10-methoxy camptothecin (10-MCPT) using LC-MS/MS and HRMS. Although solvents for mobile phase were not the same as our study, they used the same C18 column and used gradient elution of high polar to less polar mobile phase. Surprisingly, HRMS chromatograms revealed the retention times of 10-MCPT at 24.07 min and 9-MCPT at 24.53 min, which differed for 0.5 min similar to our results (Figure 3.8). 9-MCPT analog may possibly be 10-MCPT. To prove this assumption, we have to analyze the samples with standard compound of 10-MCPT, otherwise, it is necessary to use the higher techniques.

The HPLC-MS data confirms the species identification results. However, *O. pseudofasciculata* had not any HPLC-MS data to confirm that Ophi 62 is exactly this species. Among *O. harrisiana*, Ophi 18 had the remarkably high contents of 9-MCPT (Figure 3.2) and DPMI (Figure 3.6). Ophi 56 (*O. ridleyana*) was the only 10-HCPTdetecting specimen in this study (Figure 3.4). Thus, Ophi 62, Ophi 18 and Ophi 56 should be analyzed for genetic characteristics to prove they were exactly the species previously identified.

The fluctuation of CPT and CPT derivative detections in this study was possibly because of the different age of examined plants and the instability of the compounds. Some growing plants might not ready to produce secondary metabolites or produce in trace amounts below the initial detection point of the equipment. In addition, the content of any compounds produced by plant can be affected by seasonal variability, plant elicitor, and part of the plant material. The example of this case may be *Ophiorrhiza* sp. 35 that was detected only 9-MCPT and its analog in quite low amount. Another factor affecting HPLC-MS analysis was the time used in one analysis for each sample which was about an hour. Lots of samples were stayed over-night in HPLC-MS system. An increasing of crude extract concentration or hydrolysis of the compounds may occur. We decreased these possible errors by setting a temperature of the HPLC-MS system to 4°C and random injection of all samples in each time. However, the content of some compounds were fluctuation in triplicate analyses such as 10-HCPT, especially in the leaf extract.

Average contents of CPT and CPT derivatives in each *Ophiorrhiza* spp. in Figure 3.7 demonstrated that *Ophiorrhiza* plants accumulated CPT mostly in roots and in derivative forms. For *O. fucosa* and *O. ridleyana*, CPT was detected only in roots, whereas, CPT derivatives were detected in leaves and roots. It is the fact that plants possess secondary compounds to defend themselves against herbivore attacks or the manifestation of microorganisms (Sirikantaramas *et al.*, 2009). In this case, *Ophiorrhiza* plants might produce CPT mainly in root, and then CPT would be changed into watersoluble derivatives in order to be easily transported to protect the upper parts of the plants. This hypothesis may not included *O. harrisiana*, the only one species that produce CPT in both leaves and roots.

In conclusion, this is the first study which reports the detections of CPT, CPT derivatives, and chemical compounds involved in CPT biosynthesis pathway in *O. fucosa*, *O. harrisiana*, *O. plumbea*, *O. ridleyana*, and *Ophiorrhiza* sp. 35. These five *Ophiorrhiza* species could be alternative sources of CPT and CPT derivatives for anticancer research and pharmaceutical industrial production in the future. Subsequently research should focuses on a quantitative analysis of CPT and CPT derivatives for increasing the CPT-producing potential of *Ophiorrhiza* plants in Thailand.



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CHAPTER IV

SEQUENCE ANALYSES OF MATK AND TOPOISOMERASE I

In order to classify and study a coevolution in *Ophiorrhiza* spp., we constructed the molecular phylogenetic trees based on chloroplast *mat*K and nuclear *Topl* nucleotide sequences. Besides, amino acid sequences of Topl enzymes were analyzed to investigate point mutations in the CPT-producing *Ophiorrhiza* species.

4.1 Materials and Methods

From all specimens, samples from eight *Ophiorrhiza* species were examined (Table 4.1). Mostly, one sample was chosen as a representative for each species. Ophi 18, Ophi 56, and Ophi 62 were also analyzed to prove that they were exactly the species previously identified by their morphological characteristics.

4.1.1 RNA extraction and reverse transcription

Fresh leaves of each examined samples were rapidly ground with liquid nitrogen using mortar and pestle, and then extracted with RNeasy[™] Plant Mini Kit (Qiagen, Germany), following the manufacturer's protocol. Total RNA was performed on 0.8% agarose gel electrophoresis stained by ethidium bromide and visualized under UV light. A Lambda DNA-Hind III Digest (New England BioLabs Inc., USA) was used as standard molecular size. The extracted RNA was promptly kept at -80°C. Total RNA of each examined sample was converted to cDNA using SuperScript[™] III Reverse Transcriptase (Invitrogen, USA) and oligo(dT)₂₀ primer, following the manufacturer's protocol. The total cDNA of each sample was then kept at -20°C for further use in PCR amplification. For qualification and quantification of RNA and cDNA samples, gel electrophoresis method was used. Samples were loaded in 0.8% agarose gel (Bio-Rad Laboratories, USA) and run on an electrophoresis apparatus filled with 1XTAE buffer. The gel was stained with ethidium bromide solution, destained and transferred to Gel Doc[™] XR System (Bio-Rad

Laboratories, Inc., USA). The samples were visualized under UV light and photographed.

Species	Locality	Examined	Accessio	n No. (size)
	(area, province)	specimen	matK	Topl
O. fucosa	Phlio National Park,	Ophi 64	AB564412	AB564420
	Chantaburi		(1518 bp)	(2781 bp)
O. harrisiana	Than Mayom waterfall,	Ophi 27	AB564413	AB564421
-	Ko Chang, Trat		(1518 bp)	(2766 bp)
4		Ophi 18	\checkmark	_
			(1518 bp)	
O. pedunculata	Mork-Fa waterfall,	Ophi 41	AB564414	AB564422
	Chiangmai		(1518 bp)	(2766 bp)
O. plumbea	Bangpae waterfall, Phuket	Ophi 6	AB564415	AB564423
	AND SIGNS III		(1518 bp)	(2766 bp)
O. pseudofasciculata	Doi Suthep-Pui National	Ophi 37	AB564416	AB564424
	Park, Chiangmai		(1518 bp)	(2778 bp)
	Khun Kon waterfall,	Ophi 62	\checkmark	\checkmark
	Chiangrai		(1518 bp)	(2854 bp)
O. ridleyana	Mae Yom National Park,	Ophi 61	AB564417	AB564425
	Lampang		(1518 bp)	(2781 bp)
ดนร	Chae Son National Park,	Ophi 56	~	~
9	Lampang		(1518 bp)	(2832 bp)
0.990 0.	Queen Sirikit Botanical	Ophi 52	~	_
<u>ุ</u> งห เดา	Garden, Chiang Mai	3115	(1518 bp)	
O. trichocarpon	Tard Mok waterfall,	Ophi 46	AB564418	AB564426
var. glabra	Chiang Mai		(1518 bp)	(2766 bp)
<i>Ophiorrhiza</i> sp. 35	Rambhai Barni Rajabhat	Ophi 35	AB564419	AB564427
	University, Chantaburi		(1518 bp)	(2766 bp)

Table 4.1 Specimens of eight Ophiorrhiza spp. with accession numbers and size of theirfull-length matK and Topl sequences.

✓ Full-length gene was sequenced but not submitted to GenBank.

- Full-length gene was not sequenced.

4.1.2 Primers design

4.1.2.1 *matK* primers

To amplify and sequence the *mat*K gene of *Ophiorrhiza*, four primers were designed. Nucleotide *mat*K sequences of *O. pumila* (accession no. AB247150), *O. kuroiwae* (AB247256), *O. japonica* (AB257123), and *O. hayatana* (AB247255) were obtained from DDBJ/EMBL/GenBank databases. All sequences were aligned and conserved regions were selected. Details of these primers are presented in Table 4.2 and the relative positions on *mat*K gene are shown in Figure 4.1. The designed primers were synthesized by Aitbiotech Pte Ltd, Singapore.

 Table 4.2 PCR amplification primers and sequencing primers of matK gene used in this study.

	ALS/ANSIA	
Primer name	Primer sequence (5' to 3')	Direction
matKOpu-560F	TCCGTCCCCGAGGTATCTATTC	Forward
matKOpu-1188F	TGCCTCTTCCTTGCATTTATTACG	Forward
matKOpu-1693R	GCACACTTGAAAGATAGCCCATAAA	Reverse
matKOpu-2227R	ATTTCCATTTACAAGGCCTCAGAA	Reverse

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Figure 4.1 Relative positions of the PCR amplification primers and sequencing primers on *mat*K gene (1518 bp in length) of *Ophiorrhiza* spp. Arrows (→) represent forward primers. Arrows (←) represent reverse primers.

4.1.2.2 Topoisomerase I primers

Nine primers were newly designed. Nucleotide *Topl* sequences of *O. pumila* (AB372508), *O. liukiuensis* (AB372509) and *O. japonica* (AB372510) were aligned and conserved regions were selected. The designed primers were synthesized by Aitbiotech Pte Ltd, Singapore. Opstart primer was obtained from Graduate School of Pharmaceutical Sciences, Chiba University, Japan. Details of these primers are presented in Table 4.3 and the relative positions on *mat*K gene are shown in Figure 4.2.

 Table 4.3 PCR amplification primers and sequencing primers of *Topl* gene used in this study.

Primer name	Primer sequence (5' to 3')	Direction
opstart	ATGGCTGTTGAGGCCTGTA	Forward
Topl-471F	GCTAGGACTTCTGGTTGCTCA	Forward
Topl-960F	CCAATATCCCAAAGAATCAAGAA	Forward
Topl-1518F	GGTGTCAAAGAGAAGGTCGGTA	Forward
Topl-2139F	CGAAGTGGGAAAGAGGGTAGT	Forward
Topl-696R	CATTTTGTTGAACTTTTGCTGC	Reverse
Topl-1078R	TAACAGAAGCTGGTGACTTC	Reverse
Topl-1518R	TACCGACCTTCTCTTTGACACC	Reverse
Topl-1831R	GCTTTCTCATATTTCTCCTTGTCA	Reverse
Topl-2753R	CATGGCCCAGGCAAACT	Reverse
pstart Topl-	471F Topl-960F Topl-1518F To → → →	opl-2139F →→
— มหาร	topoisomerase I 2781 bp	
9	← ← ← ← Fopl-696R Topl-1078R Topl-1518R Topl-183 ⁻	■ ← 1R Topl-275

Figure 4.2 Relative positions of the PCR amplification primers and sequencing primers on *Top*I gene (2781 bp in length) of *O. pumila*. Arrows (\longrightarrow) represent forward primers. Arrows (\longleftarrow) represent reverse primers. Pin shape with white circle ($\begin{pmatrix} 0 \\ 1 \end{pmatrix}$) indicates amino acid mutation at position 530 based on *H. sapiens* TopI. Pin shape with black circle ($\begin{pmatrix} 0 \\ 1 \end{pmatrix}$) indicates mutation at position 722 based on *H. sapiens* TopI.

4.1.3 PCR amplification

The cDNA fragments encoding *mat*K and *Top*I were used as templates for PCR amplification of *mat*K and *Top*I genes using TaKaRa Ex TaqTM Polymerase (Takara Bio Inc, Japan), following the manufacturer's protocol. PCR amplification was carried out in Bio-Rad Laboratories C1000 Thermal Cycler (Bio-Rad Laboratories, Inc., USA). The PCR products were run on a 1% agarose gel with Lambda DNA/*Pst*I marker and subsequently cloned into *E. coli*.

4.1.3.1 PCR of matK gene

PCR amplification of *mat*K region was performed using 2 μ L of cDNA template in 50 μ L of reaction mixture consisting of 5 units/ μ L TaKaRa Ex Taq TM, 1X Ex Taq Buffer (including 2 mM of MgCl₂), 2.5 mM each of dNTPs mixture, and 0.2 μ M of each matKOpu-560F and matKOpu-2227R primer. The PCR cycling program started with an initial denaturation step at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 2 min, and a final extension at 72°C for 5 min, then held at 4°C. An expected size of the PCR product was 1700 bp approximately.

4.1.3.2 PCR of topoisomerase I gene

PCR amplification was performed using 2 μ L of cDNA template in 50 μ L of reaction mixture consisting of 5 units/ μ L TaKaRa Ex Taq TM, 1X Ex Taq Buffer (including 2 mM of MgCl₂), 2.5 mM each of dNTPs mixture, and 0.2 μ M of each primer. Initially, TopI-1518F and TopI-2753R primers were used to amplify a mutation region in *TopI* gene. The PCR cycling program started with an initial denaturation step at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 48°C for 30 s, extension at 72°C for 1.5 min, and a final extension at 72°C for 5 min, then held at 4°C. An expected size of the PCR product was 1200 bp approximately. Other couples of primers were used to complete a full-length gene of *Top*I using similar PCR condition with the modification of an extension time depended on fragment size.

4.1.4 Cloning and sequencing

PCR products of *mat*K and *Top*I fragments were cloned and transformed to *E. coli* DH5 α competent cells with the pGEM[®]-T Easy Vector System (Promega Corp, USA). Each PCR product (3 µL) was ligated with pGEM-T Easy Vector (1 µL) using 1 µL of T4 DNA ligase enzyme in 5 µL of 2X ligation buffer. Ligation mixture was incubated at 4°C overnight and transformed to *E. coli* DH5 α competent cells by heat shock method.

Competent cells was removed from the -80°C freezer and thawed on ice. After thawing, 100 μ L of cells was mixed with 5 μ L of ligation mixture and incubated on ice for 30 min. The cell mixture was heat shocked at 37°C for 60 s and rapidly placed on ice for 5 min and then 900 μ L of SOC media (42°C preheated) was added to the cell mixture. The cell mixture was transferred into a 15-ml tube and shacked for 1 hr at 37°C.

The recombinant clones were selected using blue/white selection technique. The LB-Amp (Luria-Bertani medium with ampicillin) plates were prepared earlier. The mixture of 2% X-Gal in DMF (40 μ L) and 100 mM IPTG (100 μ L) was spread on LB-Amp plate to prepare an X-gal plate. The recombinant *E. coli* cell mixture was plated onto the X-gal plates and placed at 37°C overnight. White colonies were randomly chosen from the overnight plates and checked for corrected size of inserts by colony PCR.

Colony PCR was performed using small amount of white colony as a template in 10 µL of reaction mixture consisting of reagents in a proportion similar to that of typical PCR amplification with T7 and SP6 primers. The PCR cycling program was the same as that of PCR product amplification, with the modification of 5 min initial denaturation time. The products of colony PCR were determined the size by gel agarose electrophoresis. The colonies inserted with expected-sized PCR products were cultured in LB-Amp broth and shacked at 37°C overnight.

Recombinant *E. coli* culture was extracted for plasmid using GenElute[™] Plasmid Miniprep Kit (Sigma-Aldrich Corp, USA), following the manufacturer's protocol.

The purified plasmids were used as templates for nucleotide sequencing by Aitbiotech Pte Ltd, Singapore.

4.1.5 Phylogenetic tree construction

The obtained *mat*K and *Top*I sequences were assembled and their consensus sequences were constructed using SeqManTM program (DNA Star Inc, USA). The nucleotide sequence data was submitted to the DDBJ/EMBL/GenBank nucleotide sequence databases with accession numbers (Table 4.1). The nucleotide sequence alignments of *mat*K and *Top*I were performed (Appendix C and D). Both separated and combined *mat*K and *Top*I sequence data-matrices were phylogenetically analyzed using PAUP* 4.0b10 program (Sinauer Assoc Inc, USA).

In the case of *mat*K sequence, *O. pumila* (accession no. AB247150), *O. kuroiwae* (AB247256), *O. japonica* (AB257123), and *O. hayatana* (AB247255) were included in the analysis with *Joosia umbellifera* (AY538396), belonging to the same family, added as an outgroup. Maximum parsimony (MP) analysis was performed using a branch-and-bound searching strategy. All characters were treated as unordered and equally weighted. Strict, semistrict and 50%-majority consensus trees of all equal MP trees were generated and compared together. Bootstrap analyses of 1000 replicates were performed with a branch-and-bound search.

MP trees of *Top*I and combined *mat*K and *Top*I data-matrices were also reconstructed with the same approach as *mat*K. *Top*I sequences of *O. pumila* (AB372508), *O. liukiuensis* (AB372509) and *O. japonica* (AB372510) were included in the *Top*I analysis with *Camptotheca acuminata* (AB372511) and *Catharanthus roseus* (AB372512) as outgroups. The combined *mat*K and *Top*I tree was midpoint-rooted without any outgroup added to the analysis.

4.1.6 Analysis of topoisomerase I amino acid

Nucleotide sequences of *topoisomerase* I of each *Ophiorrhiza* spp. were translated into amino acid sequences. Encoded amino acid sequences of *Ophiorrhiza* Topl protein were aligned using MegAlignTM program (DNA Star Inc, USA) and were compared with those of other organisms retrieved from GenBank. These additional Topl sequences were from three CPT-producing plants, *O. pumila*, *O. liukiuensis* and *Camptotheca acuminata*, and three non-CPT-producing organisms, *O. japonica*, *Catharanthus roseus* and *Homo sapiens* (NM_003286).



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4.2 Results

4.2.1 RNA determination

Total RNA extracted from leaf tissue of samples (Table 4.1) and Lambda DNA-*Hind* III marker were visualized under UV light and photographed. Figure 4.3 showed two bands of 28S and 18S ribosomal RNA (rRNA) and a smeared appearance of partially degraded RNA.



Figure 4.3 Agarose gel electrophoresis of total RNA extracted from *Ophiorrhiza* samples.

Lane M: Lambda DNA-*Hind* III marker Lane 1: *O. fucosa* (Ophi 64) Lane 2: *O. harrisiana* (Ophi 27) Lane 3: *O. pedunculata* (Ophi 41) Lane 4: *O. plumbea* (Ophi 6) Lane 5: *O. pseudofasciculata* (Ophi 37)

4.2.2 PCR and colony PCR product determination

4.2.2.1 matK gene

The PCR products of *mat*K amplified with matKOpu-560F and matKOpu-2227R primers were approximately 1700 bp in length. From agarose gel electrophoretogram, each sample showed one band of PCR product with corrected size (Figure 4.4). These were cloned and transformed to *E. coli*.



Figure 4.4 Agarose gel electrophoresis of 1700-bp *mat*K region amplified from cDNA of *Ophiorrhiza* samples.

Lane M: Lambda DNA/*Pst*I marker Lane 1: *O. fucosa* (Ophi 64) Lane 2: *O. harrisiana* (Ophi 27) Lane 3: *O. pedunculata* (Ophi 41) Lane 4: *O. plumbea* (Ophi 6) Lane 5: *O. pseudofasciculata* (Ophi 37) Lane 6: *O. ridleyana* (Ophi 61)
Eight randomly white colonies were picked from the overnight plates and checked for corrected insert size by colony PCR (Figure 4.5). The colonies inserted with 1700 bp PCR products were cultured. Plasmids were extracted using GenElute[™] Plasmid Miniprep Kit.



Figure 4.5 Colony screening for the 1700-bp size of matK region inserts. The number above each lane indicates clone number of Ophiorrhiza spp.

Lane M: Lambda DNA/Pstl marker

A: <i>O. fucosa</i> (Ophi 64)	D: <i>O. plumbea</i> (Ophi 6)
B: O. harrisiana (Ophi 27)	E: O. pseudofasciculata (Ophi 37)

C: *O. pedunculata* (Ophi 41) F: *O. ridleyana* (Ophi 61)

4.2.2.2 Topoisomerase I gene

The PCR products of *Top*I amplified with TopI-1518F and TopI-2753R primers were approximately 1250 bp in length. From agarose gel electrophoretogram (Figure 4.6), most sample showed one band of PCR product with corrected size. *O. pseudofasciculata* (lane 5) also showed two non-specific bands which were 300 bp and 800 bp in length. These were cloned and transformed to *E. coli*.



Figure 4.6 Agarose gel electrophoresis of 1250-bp *Top*I fragment amplified from cDNA of *Ophiorrhiza* samples.

Lane M: Lambda DNA/*Pst*I marker Lane 1: *O. fucosa* (Ophi 64) Lane 2: *O. harrisiana* (Ophi 27) Lane 3: *O. pedunculata* (Ophi 41) Lane 4: *O. plumbea* (Ophi 6) Lane 5: *O. pseudofasciculata* (Ophi 37) Eight randomly white colonies were picked from the overnight plates and checked for the size of inserts by colony PCR (Figure 4.7). The colonies inserted with 1250 bp PCR products were cultured. Plasmids were extracted using GenElute[™] Plasmid Miniprep Kit.



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Figure 4.7 Colony screening for the 1250-bp size of *Top*l fragment inserts. The number above each lane indicates clone number of *Ophiorrhiza* spp.

Lane M: Lambda DNA/Pstl marker

A: *O. fucosa* (Ophi 64) D: *O. plumbea* (Ophi 6)

- B: O. harrisiana (Ophi 27) E: O. pseudofasciculata (Ophi 37)
- C: O. pedunculata (Ophi 41)

4.2.3 Phylogenetic tree of matK gene

The *mat*K sequences of *O. pumila*, *O. kuroiwae*, *O. japonica*, and *O. hayatana* obtained from GenBank database were added in the analysis. *Joosia umbellifera*, belonging to the same family, was added as an outgroup. The nucleotide sequences of *mat*K were aligned using CLC Sequence Viewer program (CLC bio, Aarhus, Denmark) (Appendix C) and phylogenetically analyzed using PAUP* 4.0b10 program (Sinauer Assoc Inc, USA).

The obtained *mat*K data matrix was 1518 total characters and numbers of parsimony-informative characters were 22 (1.45%). The numbers of equally most parsimonious trees were eleven. One of the 11 maximum parsimonious trees (MPTs) was shown as a phylogram (Figure 4.8). The 50% majority consensus tree of 11 equally MPTs was constructed (Figure 4.9). The length of each MPT is 179; CI = 0.9609, RI = 0.9167 and RC = 0.8808. The 50% majority consensus tree classified *Ophiorrhiza* spp. into two major clades.



Figure 4.8 The maximum parsimonious phylogram of *mat*K gene. The length of each maximum parsimonious trees (MPTs) is 179; CI = 0.9609, RI = 0.9167 and RC = 0.8808. Numbers above the lines are branch lengths of the maximum parsimonious tree (MPT). Specimen numbers come after taxa name.



Figure 4.9 The 50% majority consensus tree of 11 equally MPTs based on the *mat*K gene. The length of each MPT is 179; CI = 0.9609, RI = 0.9167 and RC = 0.8808. Numbers above the lines are %majority between all MPTs. Numbers below the lines are %bootstrap values with 1000 replicates. Arrows indicate nodes collapsed in the strict consensus tree. Specimen numbers come after taxa name. 'A' and 'B' indicates the clade of CPT-producing and -non-producing plants.

4.2.4 Phylogenetic tree of topoisomerase I gene

The *Top*l sequences of *O. pumila*, *O. liukiuensis*, and *O. japonica* obtained from GenBank database were added in the analysis. *Camptotheca acuminata* and *Catharanthus roseus* were added as outgroups. The nucleotide sequences of *Top*l were aligned using CLC Sequence Viewer program (CLC bio, Aarhus, Denmark) (Appendix D) and phylogenetically analyzed using PAUP* 4.0b10 program (Sinauer Assoc Inc, USA).

The obtained *Topl* data matrix was 2932 total characters and numbers of parsimony-informative characters were 313 (10.68%). The numbers of equally most parsimonious trees were two. One of two MPTs was shown as a phylogram (Figure 4.10). The strict consensus tree of two equally MPTs was constructed (Figure 4.11). The length of each MPT is 1189; CI = 0.9058, RI = 0.8025 and RC = 0.7269.The strict consensus tree classified *Ophiorrhiza* spp. into two major clades.



Figure 4.10 The maximum parsimonious phylogram of *Top*I gene. The length of each MPT is 1189; CI = 0.9058, RI = 0.8025 and RC = 0.7269. Numbers above the lines are branch lengths of the MPT. Specimen numbers come after taxa name.



Figure 4.11 The strict consensus tree of two equally MPTs based on the *Top*I gene. The length of each MPT is 1189; CI = 0.9058, RI = 0.8025 and RC = 0.7269. Numbers below the lines are %bootstrap values with 1000 replicates. Specimen numbers come after taxa name. 'A' and 'B' indicates the clade of CPT-producing and -non-producing plants.

4.2.5 Phylogenetic tree of combined data of matK and topoisomerase I gene

The *mat*K and *Top*I sequences of *Ophiorrhiza* spp. were combined. The nucleotide sequences of combined *mat*K and *Top*I were aligned using CLC Sequence Viewer program (CLC bio, Aarhus, Denmark) and phylogenetically analyzed using PAUP* 4.0b10 program (Sinauer Assoc Inc, USA).

The obtained combined *mat*K and *Top*I data-matrices were 4381 total characters and numbers of parsimony-informative characters were 119 (2.72%). The single most parsimonious tree was constructed (Figure 4.12). The length of MPT is 295; CI = 0.7966, RI = 0.8137 and RC = 0.6482. The tree was midpoint-rooted without any outgroup added to the analysis. The single most parsimonious tree classified *Ophiorrhiza* spp. into two major clades.



						Leaf extract							Root extract					
						CPT	9-MCPT	9-MCPT analog	10- HCPT	PMI	DPMI	CPT	9-MCPT	9-MCPT analog	10- HCPT	PMI	DPMI	
			$\begin{array}{c} 11 \\ 5 \\ < 50 \\ 13 \\ 55 \\ 25 \\ 25 \\ 10 \\ 10 \\ \end{array}$	11 5	- <i>O. fucosa</i> 64	-	150	1to	-	_	_	+	+	+	_	+	+	
15 98		ſ		:50 13	— O. pumila	+	+		-	+	+	+	_	_	_	+	_	
		10		25	———— O. ridleyana 61	-	+	-	-	-	_	+	+	_	_	+	+	
		80		<u>10</u> 0.	harrisiana 27	+	+	+	-	+	_	+	_	+	_	+	_	
	15 87		75	8 Oph	iorrhiza sp. 35	-	+	-	-	-	-	_	+	_	_	_	_	
		1	19 O. plumbea 6			-	2 174	-	-	-	_	+	+	÷	_	+	+	
	37 O. ridleyana 56					-	+	-	+	-	_	+	+	+	+	+	+	
	13	6	6 O. pedunculata 41				202	18-14	-	ō	_	_	_	_	_	_	_	
	100	4	0. tricho	carpon 46		-	_	_	-	A.	_	_	_	_	_	_	_	
	_		<u>31</u> 100		9 O. pseudofasciculata 62	-	_	ē	_	9	_	_	_	_	_	_	_	
	13			0. pseudofasciculata 37	31	121	121	121	าก	2-	_	_	_	_	_	_		
	84 10 chang	ges	28	o	D. japonica	กร	ถ ่ เ	หา	วิเ	1៦	าลัย] -	_	-	_	_	_	

Figure 4.12 The single most parsimonious tree based on a combined *mat*K and *Topl* data matrices. The length of each MPT is 295; CI = 0.7966, RI = 0.8137 and RC = 0.6482. Numbers above the lines are branch lengths of the MPT. Numbers below the lines are %bootstrap values with 1000 replicates. The tree was midpoint-rooted without any outgroup added to the analysis. Specimen numbers come after taxa name. The results from HPLC-MS analysis of compounds detected in the leaf and root extracts of each specimen were indicated by '+' (presence) or '-' (absence) symbols.

4.2.6 The alignment of topoisomerase I amino acid sequences

According to the alignment of *Topl* nucleotide sequences (Appendix D), *O. pseudofasciculata* 62 and *O. ridleyana* 56 had 76-bp nucleotide insertion. This insertion caused numerous gaps in amino acid alignment of Topl. Therefore, Ophi 62 and Ophi 56 were excluded from Topl amino acid alignment (Appendix E). Topoisomerase I amino acid sequences of examined samples of *Ophiorrhiza* spp. (Table 4.1) were aligned with other organisms (Appendix E). The order of taxa in the alignment was arranged in clade A and B of the phylogenetic trees. The 26 positions which showed unique amino acids of each clade were reported in Figure 4.13 (A). The codons translated to these unique amino acids were reported in Figure 4.13 (B).



Figure 4.13 The 26 positions of amino acid substitutions in TopI amino acid alignment. (A) The amino acid substitutions showing two putatively different groups separated by a black line: CPT-producing organisms (below the line) and -non-producing organisms (above the line). The gray boxes indicate amino acid substitutions which have been reported in previous studies. Arrows indicate the locations of below amino acid positions in four domains of a human TopI structure (Champoux, 2001) comprising an N-terminal domain, a core domain, a linker domain, and a C-terminal domain. The numbers beneath the human TopI structure indicate domain boundaries based on amino acid sequences of human TopI. (B) The codons translated into each amino acid substitutions in (A). Hyphens indicate gaps. Red characters represent different amino acid or nucleotide sequences.

4.3 Discussion

Sequence analysis of *mat*K revealed its conserve within the genus *Ophiorrhiza*. The *mat*K genes of all *Ophiorrhiza* species and *Joosia umbellifera* were all 1518 bp in length. Parsimony-informative nucleotides were only 1.45% from 1518 bp. Figure 4.8 showed the number of different nucleotide of each taxon. The *mat*K sequences of *O. fucosa* 64, *O. ridleyana* 61, and *O. ridleyana* 56 were completely 100% identical even if they were different species. Conversely, *mat*K sequences of plant specimens in the same species were different in 1-2 bps, for instance *O. harrisiana* and *O. pseudofasciculata*. Despite the *mat*K tree did not revealed high resolution enough to divide species, the phylogenetic consensus tree of *mat*K (Figure 4.9) revealed two major clades of *Ophiorrhiza* spp. that agree with a *trnK/mat*K tree previously published (Nakamura, 2006). Clade A comprised of *O. plumbea*, *O. harrisiana*, *O. fucosa*, *O. ridleyana*, *Ophiorrhiza* sp. 35, *O. pumila*, and *O. kuroiwae*. Clade B comprised of *O. pseudofasciculata*, *O. pedunculata*, *O. trichocarpon*, *O. japonica*, and *O. hayatana*.

The *mat*K phylogenetic tree showed a correlation of *Ophiorrhiza* spp. with production of CPT and CPT derivatives. All plants in clade A can produce CPT or CPT derivatives. For instance, *O. pumila* produces CPT and 9-MCPT (Yamazaki *et al.*, 2003) and *O. kuroiwae* produces CPT and 10-MCPT (Asano *et al.*, 2009). The other *Ophiorrhiza* taxa in clade B are known to be non-CPT-producing plants, e.g. *O. hayatana* can produce only anthraquinones (Chan *et al.*, 2005). In clade B, *O. ridleyana* 52 was clustered with *O. pseudofasciculata* and separated from other *O. ridleyana* specimens. Thus, Ophi 52 is clearly not *O. ridleyana* but it is closely related with *O. pseudofasciculata*. Due to the close relationships of genetic and morphological characteristics within species of *O. harrisiana*, Ophi 18 and Ophi 27 were considered as *O. harrisiana*. From these results, Ophi 52 and Ophi 18 were excluded from *Topl* analysis.

The strict consensus tree of nuclear *Top*I gene (Figure 4.11) showed similar topology to the *mat*K tree. *O. liukiuensis* in clade A was previously reported to produce

CPT, 9-MCPT and 10-MCPT (Kitajima *et al.*, 2005). Compared with the chloroplast *mat*K tree, the nuclear *Top*I tree gave a much higher number of parsimony informative characters (10.68% of *Top*I and 1.45% of *mat*K) and showed a higher bootstrap percentage supporting the division of clade A and B (94% for *Top*I and 91% for *mat*K). Likewise, the *Top*I tree revealed higher resolution of the phylogenetic relationship between species within the tree and may suggest the evolutionary pattern in the genus *Ophiorrhiza*. The maximum parsimonious phylogram of *Top*I gene (Figure 4.10) suggests that CPT-non-producing *Ophiorrhiza* spp. (clade B) may exist before CPT-producing species (clade A). In fact, TopI enzyme is known to be a target of CPT. Therefore, the *Top*I gene of *Ophiorrhiza* could have evolved responsively to the emerging event of gene mutations for CPT production.

The single phylogenetic tree of combined *mat*K and *Top*I regions (Figure 4.12) also strongly confirmed the separation between the two groups of CPT-producing and CPT-non-producing *Ophiorrhiza* plants with a very high bootstrap value (98%). Currently, it has been no report of subgenus division in the genus *Ophiorrhiza*. In this study, there is obvious correlation between camptothecinoid detection and taxonomic positions of *Ophiorrhiza* spp. based on *mat*K and *Top*I phylogenetic trees. Thus, it is possible to divide *Ophiorrhiza* into two chemotaxonomic groups: camptothecinoid producers and camptothecinoid-non-producers.

The alignments of *mat*K and *Top*I nucleotide sequences showed several polymorphic loci which can be utilized to design molecular markers to differentiate CPT-producing and CPT-non-producing *Ophiorrhiza*. For instance, PCR-RFLP method can be developed using the different enzyme restriction sites between two groups of *Ophiorrhiza*. SCAR marker may be used if there is specific band obtained from RAPD technique.

The alignment of Topl amino acid sequences (Appendix E) showed moderate polymorphisms between *Ophiorrhiza* spp. and other plants; e.g. 68% identity between *O. plumbea* 6 and *Camptotheca acuminata*, and 63% identity between *O. harrisiana* 27

and *Catharanthus roseus*. The amino acid polymorphisms in various residue-positions (Figure 4.13) revealed the division between all examined organisms, which could be separated into two groups of CPT-producing and -non-producing organisms. Previous studies of the structure of human Topl enzyme (Champoux *et al.*, 2001; Redinbo *et al.*, 1998; Sirikantaramas *et al.*, 2008) suggested several mutated amino-acid residues that may contribute to production of a CPT-resistant Topl. The mutations of Leu-530 to lle which were found only in *O. pumila* and *O. liukiuensis*, could disrupt CPT-binding by shifting the Asp-533 that binds to CPT (numbered according to human Topl) (Sirikantaramas *et al.*, 2008) The Asn-722 which lies next to the active-site Tyr-733 has been reported to be mutated to Ser or Asp in CPT-producing plants and mutated cells which are resistant to CPT, such as some human leukemia cell-lines, yeast *Saccharomyces* spp. and viruses (Gupta *et al.*, 1995).

In this study, we found that most of CPT-producing *Ophiorrhiza* species had two amino acid mutations of Leu-530 to Ile and Asn-722 to Ser, which were identical to a previous study (Sirikantaramas *et al.*, 2008). We also found two amino acid substitutions at Met-370 and Gly-717, which have been previously reported only in yeast and human mutated cells (Wang *et al.*, 1997) but never been reported in plants. Other substituted residues (Figure 4.13) also suggest the amino acid markers in the Topl sequences of the CPT-producing or CPT derivative-producing *Ophiorrhiza* plants were comparable to the amino acid positions in four distinct domains of human Topl. These substituted residues were located near the mutated positions that affect Topl structure (Redinbo *et al.*, 1998) but had never been found in CPT-resistant human cancer cells.

The phylogenetic tree and Topl amino acid analysis results confirm that Ophi 37 and Ophi 62 were *O. pseudofasciculata*. Although *Ophiorrhiza* sp. 35 showed only CPT derivatives production but not CPT, it was placed in clade A of both *mat*K and *Top*l phylogenetic trees. Additionally, several mutated Topl residues of this plant were identical to those of CPT-producing plants. Surprisingly, *O. ridleyana* 56 was placed in clade A of both *mat*K and *Top*l phylogenetic trees but had non-mutated amino acid residues in reported critical positions. The full-length *Top*l gene of *O. ridleyana* 56 was

closely related with *O. ridleyana* 61 and other CPT-producing *Ophiorrhiza* spp. However, this plant may not have Topl mutation as a self-resistance mechanism, otherwise, there would be new point mutations which cause resistance in Topl. Hence, any further study should focus on the effect of these amino acid substitutions on protein structure, CPT-binding site, and enzyme activity of Topl.



CHAPTER V

CONCLUSION: COEVOLUTION OF TOPOISOMERASE I AND CAMPTOTHECIN PRODUCTION

Five species of *Ophiorrhiza*, *O. fucosa*, *O. harrisiana*, *O. plumbea*, *O. ridleyana*, and *Ophiorrhiza* sp. 35, out of eight species collected in this study, are reported the detections of CPT, CPT derivatives, and chemical compounds involved in CPT biosynthesis pathway. The distribution of *Ophiorrhiza* spp. suggests that *Ophiorrhiza* in Thailand had CPT production abilities mainly related to species, not habitat. The sequence analyses of chloroplast *mat*K and nuclear *Top*I genes suggest that genetic factors play an important role in determining CPT and CPT derivatives-producing properties of *Ophiorrhiza* plants. By reason that the molecular phylogenetic trees of both separated and combined *mat*K and *Top*I nucleotide sequences had similar topology and correlated with production of CPT and CPT derivatives, we conclude that *Ophiorrhiza* plants have a coevolution of *mat*K and *Top*I genes with production of CPT and CPT derivatives.

In fact, Topl enzyme is known to be a target of CPT. Therefore, the *Topl* gene of *Ophiorrhiza* could have evolved responsively to the emerging event of gene mutations for CPT production. Several amino acid residues in the *Topl* gene are preserved in CPT-producing *Ophiorrhiza* plants, probably as a self-resistance mechanism to avoid self-toxicity. Despite encoded protein of *mat*K gene is not correlated with Topl enzyme or even CPT, the phylogenetic tree exhibits the coevolution between *mat*K and CPT production. It could be possible that CPT-producing ability is established in ancestor of *Ophiorrhiza* plants in ancient times.

The alignments of *mat*K and *Top*I nucleotide sequences showed several identical positions of CPT-producing *Ophiorrhiza* which can be utilized to design molecular markers for differentiation of anticancer *Ophiorrhiza* species from non-anticancer species. For instance, PCR-RFLP method can be developed using the

different enzyme restriction sites between two groups of *Ophiorrhiza*. SCAR marker may be used if there is specific band obtained from RAPD technique.

According to the coevolution of *mat*K, *Top*I genes and production of CPT and CPT derivatives, *mat*K and *Top*I gene sequences could be utilized for prediction of CPTand CPT derivatives-production ability of any members of *Ophiorrhiza*. Such molecular techniques have greater advantages than chemical techniques to suggest production of CPT and CPT derivatives in *Ophiorrhiza* spp. For instance, we can use this molecular technique for plants that produce trace amounts of CPT at levels below the initial detection point of the equipment. Likewise, some plants may produce only CPT derivatives that are more sensitive than the parental CPT molecule and some of this amount may be lost through the material processing technique. Moreover, our molecular analysis is not affected by seasonal variability, plant elicitor, or stage and part of the plant material. If any plant of the genus is analyzed and placed in clade A of the *mat*K and *Top*I phylogenetic trees, this would indicate a close relationship to CPT-producing plants and thus they may produce some amount of CPT or CPT derivatives.

Additionally, the mutation points in Topl amino acid sequences also supported the nucleotide phylogenetic trees on the prediction of CPT-producing ability in *Ophiorrhiza*. The results in the present study thus strengthen our hypothesis that members of the genus *Ophiorrhiza* producing CPT or CPT derivatives should have specific mutations in the *Topl* gene. CPT-producing *O. ridleyana* 56, which placed in clade A of both *mat*K and *Topl* phylogenetic trees, had non-mutated amino acid residues in reported critical positions. This disagreeable result brings into question that there would be other unreported point mutations in Topl which cause CPT-resistance. This study is fundamental research toward anticancer development from natural resources based on CPT and CPT derivatives. Any further study should focuses on the effect of amino acid substitutions on protein structure, CPT-binding site and enzyme activity of Topl. The anticancer-producing *Ophiorrhiza* species should be used as alternative sources of anticancer research and pharmaceutical industrial production development. Subsequently research should focuses on a quantitative analysis of CPT and CPT derivatives production. Tissue culture technique is also interesting to be utilized for increasing the CPT-producing potential of *Ophiorrhiza* plants in Thailand. Our finding could provide useful information toward recognition of the point mutations in CPT-resistant cancer patients in the future.



REFERENCES

- Aiyama, R., Nagai, H., Nokata, K., Shinohara, C., and Sawada, S., 1988. A camptothecin derivative from *Notapodytes foetida*. <u>Phytochemistry</u> 27: 3663-3664.
- Arbain, D., Putra, D. P., and Sargent, M. V. 1993. The Alkaloids of *Ophiorrhiza filistipula* Australian Journal of Chemistry 46(7): 977-985.
- Arisawa, M., Gunasekera, S. P., Cordell, G. A., and Farnsworth, N. R. 1981. Plant anticancer agents XXI. Constituents of *Merrilliodendron megacarpum*. <u>Planta</u> <u>medica</u> 43: 404-407.
- Asano, T., Sudo, H., Yamazaki, M., and Saito, K. 2009. Camptothecin production by *in vitro* cultures and plant regeneration in *Ophiorrhiza* species. <u>Methods in molecular biology</u> 547: 337-345.
- Benedetti, P., Fiorani, P., Capuani, L., and Wang, J. C. 1993. Camptothecin resistance from a single mutation changing glycine 363 of human DNA topoisomerase I to cysteine. <u>Cancer research</u> 53(18): 4343-4348.
- Bodley, A. L., and Shapiro, T. A. 1995. Molecular and cytotoxic effects of camptothecin, a topoisomerase I inhibitor, on trypanosomes and Leishmania. <u>Proceedings of the National Academy of Sciences of the United States of</u> <u>America</u> 92(9): 3726-3730.
- Carbonero, G. R., and Supko, J. G. 2002. Current perspectives on the clinical experience pharmacology, and continued development of the camptothecins. <u>Clinical cancer research</u> 8: 641-661.
- CBOL Plant Working Group. 2009. A DNA barcode for land plants. <u>Proceedings of the</u> <u>National Academy of Sciences of the United States of America</u> 106(31): 12794-12797.
- Chamchumroon, V., and Puff, C. 2003. The Rubiaceae of Ko Chang, south-eastern Thailand. <u>Thai Forest Bulletin (Botany)</u> 31: 13-26.
- Chamchumroon, V., Chayamarit, K., and Puff, C. 2005. <u>Rubiaceae of Thailand. A</u> <u>pictorial guide to indigenous and cultivated genera</u>. Bangkok: The Forest Herbarium, National Park, Wildlife and Plant Conservation Department.

- Champoux, J. J. 1981. DNA is linked to the rat liver DNA nicking-closing enzyme by a phosphodiester bond to tyrosine. <u>The Journal of biological chemistry</u> 256(10): 4805-4809.
- Champoux, J. J. 2001. DNA topoisomerases: structure, function, and mechanism. <u>Annual review of biochemistry</u> 70: 369-413.
- Chan, H. H., Li, C. Y., Damu, A. G., and Wu, T. S. 2005. Anthraquinones from *Ophiorrhiza hayatana* OHWI. <u>Chemical & pharmaceutical bulletin</u> 53(10): 1232-1235.
- Chou, F. S., Yang, C. K., and Liao, C. K. 2006. Taxonomic Status of *Ophiorrhiza michelloides* (Masam.) X. R. Lo (Rubiaceae) in Taiwan. <u>Taiwania</u> 51(2): 143-147.
- Chourpa, I., Millot, J. M., Sockalingum, G. D., Riou, J. F., and Manfait, M. 1998. Kinetics of lactone hydrolysis in antitumor drugs of camptothecin series as studied by fluorescence spectroscopy. <u>Biochimica et Biophysica Acta</u> 1379: 353-366.
- Chrencik, J. E., Staker, B. L., Burgin, A. B., Pourquier, P., Pommier, Y., Stewart, L., and Redinbo, M. R. 2004. Mechanisms of Camptothecin Resistance by Human Topoisomerase I Mutations. <u>Journal of molecular biology</u> 339: 773-784.
- Ciddi, V., and Shuler, M. L. 2000. Camptothecin from callus cultures of *Nothapodytes foetida*. <u>Biotechnology letters</u> 22: 129-132.
- Dai, J. R., Cardellina, J. H., and Boyd, M. R. 1999. 20-O-β-Glucopyranosyl camptothecin from *Mostuea brunonis*: a potential camptothecin pro-drug with improved solubility. Journal of natural products 62: 1427-1429.
- Dancey, J., and Eisenhauer, E. A. 1996. Current perspectives on camptothecins in cancer treatment. <u>British Journal of Cancer</u> 74: 327-338.
- Darwin, C. 1859. On the origin of species. London: John Murray.
- Darwin, S. V. 1976. The pacific species of *Ophiorrhiza* L. (Rubiaceae). Lyonia 1(2): 47-102.
- Das, B., and Madhusudhan, P. 1999. Isolation, Characterization and Chemoenzymatic synthesis of 9-methoxy-20-(S)-mappicine, a new constituent of *Nothapodytes foetida*. <u>Natural Product Letters</u> 14(2): 135-140.

- Del Bino, G., Lassota, P., and Darzynkiewicz, Z. 1991. The S-phase cytotoxicity of camptothecin. <u>Experimental Cell Research</u> 193: 27-35.
- Fertala, J., Vance, J. R., Pourquier, P., Pommier, Y., and Bjornsti, M. A. 2000. Substitutions of Asn-726 in the active site of yeast DNA topoisomerase I define novel mechanisms of stabilizing the covalent enzyme-DNA intermediate. <u>The</u> <u>journal of biological chemistry</u> 275 (20): 15246-15253.
- Flowers, J. L., Hoffman, R. M., Driscoll, T. A., Wall, M. E., Wani, M. C., Manikumar, G., Friedman, H. S., Dewhirst, M., Colvin, O. M., and Adams, D. J. 2003. The activity of camptothecin analogues is enhanced in histocultures of human tumors and human tumor xenografts by modulation of extracellular pH. <u>Cancer</u> <u>chemotherapy and pharmacology</u> 52: 253-261.
- Frank, S. A. 1993. Coevolutionary genetics of plants and pathogens. <u>Evolutionary</u> <u>Ecology</u> 7: 45-75.
- Fulzele, D. P., Satdive, R. K., and Pol, B. B. 2001. Growth and production of camptothecin by cell suspension cultures of *Nothapodytes foetida*. <u>Planta</u> <u>medica</u> 67: 150-152.
- Gloor, G. B., Martin, L. C., Wahl, L. M., and Dunn, S. D. 2005. Mutual information in protein multiple sequence alignments reveals two classes of coevolving positions. <u>Biochemistry</u> 44(19): 7156-7165.
- Goh, C. S., Bogan, A. A., Joachimiak, M., Walther, D., and Cohen, F. E. 2000. Coevolution of proteins with their interaction partners. <u>Journal of molecular biology</u> 299: 283-293.
- González, R. D., Pertejo, Y. P., Redondo, C. M., Pommier, Y., Balaña-Fouce, R., and Reguera, R. M. 2007. Structural insights on the small subunit of DNA topoisomerase I from the unicellular parasite *Leishmania donovani*. <u>Biochimie</u> 89: 1517-1527.
- Govindachari, T. R., and Viswanathan, N. 1972. Alkaloids of *Mappia foetida*. <u>Phytochemistry</u> 11(12): 3529-3531.

- Gunasekera, S. P., Badawi, M. M., Cordell, G. A., Farnsworth, N. R., and Chitnis, M. 1979. Plant anticancer agents X. Isolation of camptothecin and 9-methoxy camptothecin from *Ervatamia heyneana*. <u>Journal of natural products</u> 42: 475-477.
- Gupta, M., Fujimori, A., and Pommier, Y. 1995. Eukaryotic DNA topoisomerases I. <u>Biochimica et biophysica acta</u> 1262(1): 1-14.
- Hausner, G., Olson, R., Simon, D., Johnson, I., Sanders, E. R., Karol, K. G., McCourt, R.
 M., and Zimmerly, S. 2006. Origin and evolution of the chloroplast *trn*K (*mat*K) intron: A model for evolution of group II intron RNA structures. <u>Molecular biology</u> and evolution 23(2): 380-391.
- Herrera, C. M. 1985. Determinants of plant-animal coevolution: the case of mutilalistic dispersal of seeds by vertebrates. <u>Oikos</u> 44: 132-141.
- Hilu, K., Borsch, T., Müller, K., Soltis, D. E., Soltis, P. S., Savolainen, V., Chase, M. W., Powell, M. P., Alice, L. A., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T. A. B., Rohwer, J. G., Campbell, C., and Chatrou, L. W. 2003. Angiosperm phylogeny based on *matK* sequence information. <u>American Journal of Botany</u> 90(12): 1758-1776.
- Hsiang, Y. H., Hertzberg, R., Hecht, S., and Liu, L. F. 1985. Camptothecin induces protein-linked DNA breaks *via* mammalian DNA topoisomerase I. <u>The Journal of</u> <u>biological chemistry</u> 260: 14873-14878.
- Hsiang, Y. H., Liu, L. F., Wall, M. E., Wani, M. C., Nicholas, A. W., Manikumar, G., Kirshenbaum, S., Silber, R., and Potmesil, M. 1989. DNA topoisomerase Imediated DNA cleavage and cytotoxicity of camptothecin analogues. <u>Cancer</u> <u>research</u> 49: 4385-4389.
- Kingsbury,W. D., Boehm, J. C., Jakas, D. R., Holden, K. G., Hecht, S. M., Gallagher, G., Caranfa, M. J., McCabe, F. L., Faucette, L. F., Johnson, R. K., and Hertzberg, R.
 P. 1991. Synthesis of water-soluble (aminoalkyl) camptothecin analogues: inhibition of topoisomerase I and antitumor activity. <u>Journal of medicinal</u> <u>chemistry</u> 34: 98-107.

- Kitajima, M., Fujii, N., Yoshino, F., Sudo, H., Saito, K., Aimi, N., and Takayama H. 2005.
 Camptothecins and two new monoterpene glucosides from *Ophiorrhiza liukiuensis*. <u>Chemical & pharmaceutical bulletin</u> 53(10): 1355-1358.
- Kitajima, M., Masumoto, S., Takayama, H., and Aimi, N. 1998. Isolation and partial synthesis of 3(*R*)- and 3(*S*)-deoxypumiloside; structural revision of the key metabolite from the camptothecin producing plant, *Ophiorrhiza pumila*. <u>Tetrahedron letters</u> 38: 4255-4258.
- Klausmeyer, P., McCloud, T. G., Melillo, G., Scudiero, D. A., Cardellina, J. H. 2nd, and Shoemaker, R. H. 2007. Identification of a new natural camptothecin analogue in targeted screening for HIF-1αinhibitors. <u>Planta medica</u> 73(1): 49-52.
- Kubota, N., Kanzawa, F., Nishio, K., Takeda. Y., Ohmori, T., Fujiwara, Y., Terashima, Y., and Saijo, N. 1992. Detection of topoisomerase I gene point mutation in CFT-11 resistant lung cancer cell line. <u>Biochemical and biophysical research communications</u> 188: 571-577.
- Kudoh, H., Sugawara, T., Wu, S., and Murata, J. 2001. Morph-specific correlations between floral traits in a distylous *Ophiorrhiza napoensis* (Rubiaceae) population in southern China. <u>Journal of Tropical Ecology</u> 17:719-728.
- Li, S., Yi, Y., Wang, Y., Zhang, Z., and Beasley, R. S. 2002. Camptothecin accumulation and variations in *Camptotheca*. <u>Planta medica</u> 68: 1010-1016.
- López-Meyer, M., Nessler, C. L., and McKnight, T. D. 1994. Sites of accumulation of the antitumor alkaloid camptothecin in *Camptotheca acuminata*. <u>Planta medica</u> 60: 558-560.
- Lorence, A., and Nessler, C. L. 2004. Camptothecin, over four decades of surprising findings. <u>Phytochemistry</u> 65(20): 2735-2749.
- Lorence, A., Medina-Bolivar, F., and Nessler, C. L. 2004. Camptothecin and 10hydroxycamptothecin from *Camptotheca acuminata* hairy roots. <u>Plant cell</u> <u>reports</u> 22: 437-441.

- Lu, Y., Wang, H., Wang, W., Qian, Z., Li, L., Wang, J., Zhou, G., and Kai, G. 2008.
 Molecular characterization and expression analysis of a new cDNA encoding strictosidine synthase from *Ophiorrhiza japonica*. <u>Molecular biology reports</u> 36(7): 1845-1852.
- Martin, K. P., Zhang, C. L., Hembrom, M. E., Slater, A., and Madassery J. 2008. Adventitious root induction in *Ophiorrhiza prostrata*: a tool for the production of camptothecin (an anticancer drug) and rapid propagation. <u>Plant biotechnology</u> <u>reports</u> 2: 163-169.
- Matile, P. 1976. Localization of alkaloids and mechanism of their accumulation in vacuoles of Chelidonium majus laticifers. <u>Nova acta Leopoldina</u> 7: 139-156.
- Muggia, F. M., Creaven, P. J., Hansen, H. H., Cohen, M. H., and Selawry, O. S. 1972.
 Phase I clinical trial of weekly and daily treatment with camptothecin (NSC-100880): correlation with preclinical studies. <u>Cancer Chemotherapy Report</u> 56: 515-521.
- Nakamura, K., Chung, S. W., Kokubugata, G., Denda, T., and Yokota, M. 2006. Phylogenetic systematics of the monotypic genus *Hayataella* (Rubiaceae) endemic to Taiwan. <u>Journal of plant research</u> 119: 657-661.
- Nakamura, K., Denda, T., Kameshima, O., and Yokota, M. 2007. Breakdown of distyly in a tetraploid variety of *Ophiorrhiza japonica* (Rubiaceae) and its phylogenetic analysis. <u>Journal of plant research</u> 120:501-509.
- Nakamura, K., Denda, T., Kokubugata, G., Suwa, R., Yang, T. Y. A., Peng, C. I., and Yokota, M. 2010. Phylogeography of *Ophiorrhiza japonica* (Rubiaceae) in continental islands, the Ryukyu Archipelago, Japan. <u>Journal of Biogeography:</u> 1365-2699.
- Narkunan, K., Chen, X., Kochat, H., and Hausheer, F. 2009. C-10 substituted camptothecin analogs. <u>United States Patent Application Publication</u> 11/974, 756.
- Osheroff, N. 1998. Preface DNA topoisomerases. <u>Biochimica et Biophysica Acta</u> 1400: 1-2.

- Pantazis, P., Han, Z., Chatterjee, D., and Wyche, J. 1999. Water-insoluble camptothecin analogues as potential antiviral drugs. <u>Journal of biomedical science 6</u>: 1-7.
- Pellegrini, M., Marcotte, E. M., Thompson, M. J., Eisenberg, D., and Yeates, T. O. 1999. Assigning protein functions by comparative genome analysis: protein phylogenetic profiles. <u>Proceedings of the National Academy of Sciences of the</u> <u>United States of America</u> 96: 4285-4288.
- Pommier, Y., Pourquoer, P., Fan, Y., and Strumberg, D. 1998. Mechanism of action of eukaryotic DNA topoisomerase I and drugs targeted to the enzyme. <u>Biochimica</u> <u>et Biophysica Acta</u> 1400: 83-105.
- Puff, C. 2007. <u>Flora of Thailand: Rubiaceae</u>[Online]. Available from: (http://homepage. univie.ac.at/christian.puff/FTH-RUB/FTHRUB_HOME.htm)[2010, September 30]
- Puff, C., and Chayamarit, K. 2006. <u>Plants of Khao Yai National Park.</u> Bangkok: The National Park Office, National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment.
- Puff, C., and Chayamarit, K. 2007. <u>Plants of Doi Inthanon National Park.</u> Bangkok: The National Park Office, National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment.
- Putiyanan, S., and Maxwell, J. F. 2007. Survey and Herbarium Specimens of Medicinal Vascular Flora of Doi Chiang Dao. <u>Chiang Mai University Journal of Natural</u> <u>Sciences</u> 6(1): 159-167.
- Rasheed, Z. A., and Rubin, E. H. 2003. Mechanisms of resistance to topoisomerase Itargeting drugs. <u>Oncogene</u> 22: 7296-7304.
- Redinbo, M. R., Stewart, L., Kuhn, P., Champoux, J. J., and Hol, W. G. 1998. Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. <u>Science</u> 279(5356): 1504-1513.
- Rehman, S., Shawl, A. S., Kour, A., Sultan, P., Ahmad, K., Khajuria, R., and Qazi, G. N.
 2009. Comparative studies and identification of camptothecin produced by an endophyte at shake flask and bioreactor. <u>Natural Product Research</u> 23(11): 1050-1057.

- Reid, R. J. D., Benedetti, P., and Bjornsti, M. A. 1998. Yeast as a model organism for studying the actions of DNA topoisomerae-targeted drugs. <u>Biochimica et</u> <u>Biophysica Acta</u> 1400: 289-300.
- Roja, G. 2006. Comparative studies on the camptothecin content from *Nothapodytes foetida* and *Ophiorrhiza* species. <u>Natural Product Research</u>: 20(1): 85-88.
- Roja, G. 2008. Micropropagation and production of Camptothecin from *in vitro* plants of *Ophiorrhiza rugosa* var. *decumbens*. <u>Natural Product Research</u> 22(12): 1017-1023.
- Roja, G., and Heble, M. R. 1994. The quinoline alkaloids camptothecin and 9-methoxy camptothecin from tissue cultures and mature trees of *Nothapodytes foetida*. <u>Phytochemistry</u> 36: 65-66.
- Rubin, E., Panayotis, P., Bharti, A., Toppmeyer, D., Giovanella, B., and Kufe, D. 1994.
 Identification of a mutant human topoisomerase I with intact catalytic activity and resistance to 9-nitro-camptothecin. <u>The Journal of biological chemistry</u> 269: 2433-2439.
- Saito, K., Sudo, H., Yamazaki, M., Koseki-Nakamura, M., Kitajima, M., Takayama, H., and Aimi, N. 2001. Feasible production of camptothecin by hairy root culture of *Ophiorrhiza pumila*. <u>Plant cell reports</u> 20: 267-271.
- Sakato, K., Tanaka, H., Mukai, N., and Misawa, M. 1974. Isolation and identification of camptothecin from cells of *Camptotheca acuminata* suspension cultures. <u>Agricultural and biological chemistry</u> 38: 217-218.
- Santisuk, T., Chayamarit, K., Pooma, R., and Suddee, S. 2006. Thailand red data: plants. <u>Office of Natural Resources and Environmental Policy and Planning</u> <u>Biodiversity Series</u> 17: 41
- Sawada, S., Okazima, S., Aiyama, R., Nokata, K., Furuta, T., Yokokura, T., Sugino, E., Yamaguchi, K., and Miyasaka, T. 1991. Synthesis and antitumor activity of 20(S)-camptothecin derivatives: carbamate-linked, water-soluble derivatives of 7-ethyl-10-hydroxycamptothecin. <u>Chemical & pharmaceutical bulletin</u> 39: 1446-1450.

- Schanzer, I. A. 2004. Systematic notes on *Ophiorrhiza trichocarpon* Blume (Rubiaceae) and some related species. <u>Thai Forest Bulletin (Botany)</u> 32: 132-145.
- Schanzer, I. A. 2005. Three new species of *Ophiorrhiza* (Rubiaceae-Ophiorrhizeae) from Thailand. <u>Thai Forest Bulletin (Botany)</u> 33: 161-170.
- Shweta, S., Zuehlke, S., Ramesha, B. T., Priti, V., Kumar, P. M., Ravikanth, G., Spiteller,
 M., Vasudeva, R., and Shaanker, R. U. 2010. Endophytic fungal strains of *Fusarium solani*, from *Apodytes dimidiata* E. Mey. ex Arn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin.
 <u>Phytochemistry</u> 71(1): 117-122.
- Sirikantaramas, S., Sudo, H., Asano, T., Yamazaki, M., and Saito, K. 2007. Transport of camptothecin in hairy roots of *Ophiorrhiza pumila*. <u>Phytochemistry</u> 68: 2881-2886.
- Sirikantaramas, S., Yamazaki, M., and Saito, K. 2008. Mutations in topoisomerase I as a self-resistance mechanism coevolved with the production of the anticancer alkaloid camptothecin in plants. <u>Proceedings of the National Academy of Sciences of the United States of America</u> 105(18): 6782-6786.
- Sirikantaramas, S., Yamazaki, M., and Saito, K. 2009. A survival strategy: the coevolution of the camptothecin biosynthetic pathway and self-resistance mechanism. <u>Phytochemistry</u> 70: 1894-1898.
- Smitinand, T. 2001. <u>Thai plant names</u>. Bangkok: The Forest Herbarium, Royal Forest Department.
- Socolich, M., Lockless, S. W., Russ, W. P., Lee, H., Gardner, K. H., and Ranganathan, R. 2005. Evolutionary information for specifying a protein fold. <u>Nature</u> 437: 512-518.
- Srinivas, K. V. N. S., and Das, B. 2003. 9-Methoxy-20-O-acetylcamptothecin, a minor new alkaloid from *Nothapodites foetida*. <u>Biochemical Systematics and Ecology</u> 31: 85-87.
- Strumberg, D., Pilon, A. A., Smith, M., Hickey, R., Malkas, L., and Pommier, Y. 2000. Conversion of topoisomerase I cleavage complexes on the leading strand of

ribosomal DNA into 5'-phosphorylated DNA double-strand breaks by replication runoff. <u>Molecular and. Cellular Biology</u> 20: 3977-3987.

- Süel, G. M., Lockless, S. W., Wall, M. A., and Ranganathan, R. 2003. Evolutionarily conserved networks of residues mediate allosteric communication in proteins. <u>Nature structural biology</u> 10: 59-69.
- Tafur, S., Nelson, J. D., DeLong, D. C., and Svoboda, G. H. 1976. Antiviral components of *Ophiorrhiza mungos*. Isolation of camptothecin and 10-methoxycamptothecin. <u>Lloydia</u> 39(4): 261-262.
- Takayama, H., Watanabe, A., Hosokawa, M., Chiba, K., Satoh, T., and Aimi, N. 1998. Synthesis of a new class of camptothecin derivatives, the long-chain fatty acid esters of 10-hydroxycamptothecin, as a potent prodrug candidate, and their *in vitro* metabolic conversion by carboxylesterases. <u>Bioorganic & medicinal</u> <u>chemistry letters</u>. 8(5): 415-418.
- Tamura, H., Kohchi, C., Yamada, R., Ikeda, T., Koiwai, O., Patterson, E., Keene, J., Okada, K., Kjeldsen, E., Nishikawa, K., and Andoh, T. 1990. Molecular cloning of a cDNA of a camptothecin-resistant human DNA topoisomerase I and identification of mutation sites. <u>Nucleic Acids Research</u> 19: 69-75.
- Tan, H., and Rao, A. N. 1981. Vivipary in *Ophiorrhiza tomentosa* Jack (Rubiaceae). <u>Biotropica</u> 13(3): 232-233.
- Urasaki, Y., Laco, G. S., Pourquier, P., Takebayashi, Y., Kohlhagen, G., Gioffre, C., Zhang, H., Chatterjee, D., Pantazis, P., and Pommier, Y. 2001. Characterization of a novel topoisomerase I mutation from a camptothecin-resistant human prostate cancer cell line. <u>Cancer research</u> 61(5):1964-1969.
- van Hengel, A. J., Harkes, M. P., Wichers, H. J., Hesselink, P. G. M., and Buitelaar, R. M.
 1992. Characterization of callus formation and camptothecin production by cell
 lines of *Camptotheca acuminata*. <u>Plant cell, tissue and organ culture</u> 28: 11-18.

- Vladu, B., Woynarowski, J. M., Manikumar, G., Wani, M. C., Wall, M. E., Von Hoff, D. D., and Wadkins, R. M. 2000. 7- and 10-substituted camptothecins: dependence of topoisomerase I-DNA cleavable complex formation and stability on the 7- and 10-substituents. <u>Molecular pharmacology</u> 57(2): 243-251.
- Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T., and Sim, G. A. 1966.
 Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. Journal of the American Chemical Society 88: 3888-3890.
- Wang, J. C. 1996. DNA topoisomerases. <u>Annual Review of Biochemistry</u> 65: 635-692.
- Wang, L. F., Ting, C. Y., Lo, C. K., Su, J. S., Mickley, L. A., Fojo, A. T., Whang-Peng, J., and Hwang, J. 1997. Identification of mutations at DNA topoisomerase I responsible for camptothecin resistance. <u>Cancer research</u> 57(8): 1516-1522.
- Wang, X., Zhou, X., and Hecht, S.M. 1999. Role of the 20-hydroxyl group in camptothecin binding by the topoisomerase I-DNA binary complex. <u>Biochemistry</u> 38: 4374-4381.
- Wani, M. C., and Wall, M. E. 1969. Plant antitumor agents. II. Structure of two new alkaloids from *Camptotheca acuminata*. <u>The Journal of organic chemistry</u> 34: 1364-1367.
- Wiedenfeld, H., Furmanowa, M., Roeder, E., Guzewska, J., and Gustowski, W. 1997.
 Camptothecin and 10-hydroxycamptothecin in callus and plantlets of *Camptotheca acuminata*. <u>Plant cell, tissue and organ culture</u> 49: 213-218.
- Wink, M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. <u>Phytochemistry</u> 64: 3-19.
- Woo, M. H., Vance, J. R., Marcos, A. R., Bailly, C., and Bjornsti, M. A. 2002. Active site mutations in DNA topoisomerase I distinguish the cytotoxic activities of camptothecin and the indolocarbazole, rebeccamycin. <u>The Journal of biological chemistry</u> 277(6): 3813-3822.
- Wu, T. S., Leu, Y. L., Hsu, H. C., Ou, L. F., Chen, C. C., Chen, C. F., Ou, J. C., and Wu,
 Y. C. 1995. Constituents and cytotoxic principles of *Nothapodytes foetida*.
 <u>Phytochemistry</u> 39: 383-385.

- Yamazaki, Y., Urano, A., Sudo, H., Kitajima, M., Takayama, H., Yamazaki, M., Aimi, N., and Saito, K. 2003. Metabolite profiling of alkaloids and strictosidine synthase activity in camptothecin producing plants. <u>Phytochemistry</u> 62: 461-470.
- Yamazaki, Y., Kitajima, M., Arita, M., Takayama, H., Sudo, H., Yamazaki, M., Aimi, N., and Saito, K. 2004. Biosynthesis of camptothecin. In silico and *in vivo* tracer study from [1-¹³C]glucose. <u>Plant physiology</u> 134(1): 161-70.
- Yip, K. Y., Patel, P., Kim, P. M., Engelman, D. M., McDermott, D., and Gerstein, M. 2008.
 An integrated system for studying residue coevolution in proteins.
 <u>Bioinformatics</u> 24(2):290-292.
- Zhang, R., Li, Y., Cai, Q., Liu, T., Sun, H., and Chambless, B. 1998. Preclinical pharmacology of the natural product anticancer agent 10-hydroxycamptothecin, an inhibitor of topoisomerase I. <u>Cancer chemotherapy and pharmacology</u> 41(4): 257-267.
- Zhou, B. N., Hoch, J. M., Johnson, R. K., Mattern, M. R., Eng, W. K., Ma, J., Hecht, S. M., Newman, D. J., and Kingston, D. G. I. 2000. Use of COMPARE analysis to discover new natural product drugs: isolation of camptothecin and 9methoxycamptothecin from a new source. <u>Journal of natural products</u> 63: 1273-1276.

APPENDICES

ศูนย์วิทยทรัพยากร จุฬาลงกรณ์มหาวิทยาลัย

Ophiorrhiza specimens

APPENDIX A



Figure A1 *Ophiorrhiza fucosa* Hance: a) habitat; b) and c) inflorescence; d) peduncle in fruit; e) upper leaf surface; f) lower leaf surface.



Figure A2 *Ophiorrhiza harrisiana* B. Heyne ex Hook. f.: a) whole plant; b) habitat; c), d), e), and f) inflorescence; g) and i) upper leaf surface; h) and j) lower leaf surface.


Figure A3 Stereo Microscope images of *Ophiorrhiza harrisiana* B. Heyne ex Hook. f.: a) and e) inflorescence (7X); b) and f) brevistylous flower (7X, 10X); c) longistylous flower (10X); d) hair ring of c) (45X); g), h) and i) fruit (7X). X indicates magnification of image.



Figure A4 Ophiorrhiza pedunculata Schanzer (O. hispidula Wall. ex G.Don var. longipedunculata Craib): a) habitat; b) habit; c) inflorescence; d) peduncle; e) flowers.



Figure A5 Ophiorrhiza plumbea Craib: a) and b) habitat; c) inflorescence;d) longistylous flower; e) brevistylous flower; f) fruit.



Figure A6 Ophiorrhiza pseudofasciculata Schanzer, Ophi 37: a) whole plant; b) inflorescence; c) and d) flower; e) upper leaf surface; f) lower leaf surface.



Figure A7 *Ophiorrhiza pseudofasciculata* Schanzer, Ophi 62: a) habit; b) inflorescence; c) and d) enlarged inflorescence; e) upper leaf surface.

จุฬาลงกรณ์มหาวิทยาลัย



Figure A8 Ophiorrhiza ridleyana Craib: a) habit; b) flower buds; c) inflorescence; d) brevistylous flower; e) longistylous flower; f) fruits.



Figure A9 *Ophiorrhiza trichocarpon* Blume var. *glabra* Schanzer: a) habitat; b) habit; c) inflorescence; d) enlarged flower; e) peduncle in fruit; f) enlarged fruits.



Figure A10 *Ophiorrhiza sp.* 35: a) habit; b) whole plant; c) bruised whole plant; d) inflorescence; e) bruised inflorescence; f) longistylous flower; g) fruits; h) bruised fruits.



HPLC-DAD chromatograms, UV spectra and mass spectra of standard compounds



Figure B1 HPLC-DAD chromatograms, UV spectra and mass spectra of 10 ng/10 μ L camptothecin standard: (A) HPLC-DAD chromatogram monitored at 254 nm; (B) UV spectrum and (C) mass spectrum.



Figure B2 HPLC-DAD chromatograms, UV spectra and mass spectra of 100 ng/10 μL9-methoxy camptothecin standard: (A) HPLC-DAD chromatogram monitored at 254 nm;(B) UV spectrum and (C) mass spectrum.



Figure B3 HPLC-DAD chromatograms, UV spectra and mass spectra of 1 μg/μL
10-hydroxy camptothecin standard: (A) HPLC-DAD chromatogram monitored at 254 nm;
(B) UV spectrum and (C) mass spectrum.



Figure B4 HPLC-DAD chromatograms, UV spectra and mass spectra of 100 ng/10µL pumiloside standard: (A) HPLC-DAD chromatogram monitored at 254 nm; (B) UV spectrum and (C) mass spectrum.



Figure B5 HPLC-DAD chromatograms, UV spectra and mass spectra of 1µg/µL 3(*S*)-deoxy pumiloside standard: (A) HPLC-DAD chromatogram monitored at 254 nm; (B) UV spectrum and (C) mass spectrum.



Figure B6 HPLC-DAD chromatograms, UV spectra and mass spectra of $1\mu g/\mu L$ chaboside standard: (A) HPLC-DAD chromatogram monitored at 254 nm; (B) UV spectrum. Mass spectrum cannot be detected.



Figure B7 HPLC-DAD chromatograms, UV spectra and mass spectra of 500 ng/µL mappicine standard: (A) HPLC-DAD chromatogram monitored at 254 nm; (B) UV spectrum and (C) mass spectrum.

APPENDIX C

The alignment of *mat*K nucleotide sequences of *Ophiorrhiza* species. Dots represent identical nucleotides. Red characters represent different nucleotides.

		20		40		60		80
O. plumbea 6	ATGGAGAAAA	TCCAAAGATA	TTTACAGCTT	GATAGATCTC	AACAACACGG	сттттатат	CCACTTATCT	TTCAGGAGTA 80
O. harrisiana 18 O. harrisiana 27								
O. fucosa 64								
O. ridleyana 56								80
Opniormiza sp. 35 O. pumila								
O. kuroiwae								
O. ridleyana 52								80
O. pseudofasciculata 62 O. pedunculata 41								
O. trichocarpon 46							т.	
O. japonica O. hayatana								
Joosia umbellifera	GG	G						
O. plumbea 6	TGTTTATGGA	CTTGCTCATG	ATCATAGGTT	AAACCGATCT	AGTITGITGG	AAAATCCAGG	TTATGACAAA	AAATCCAGTT 16
O. harrisiana 18								
O. fucosa 64								
O. ridleyana 61 O. ridleyana 56			•••••	•••••				
Ophiorrhiza sp. 35				A			G	
O. pumia O. kuroiwae								
O. pseudofasciculata 37			<u>T</u>					
O. pseudofasciculata 62			т.					
O. pedunculata 41 O. trichocarpon 46			· · · · · · · · · · · · · · · · · · ·	•••••••				
O. japonica			<u>T</u>					
Joosia umbellifera	. A C .			Ť	.т		GT	C 16
		180		200		220		240
O. plumbea 6 O. harrisiana 18	TCCTAATTGT	GAAACGTTTA	ATTACTCGAA	TGTATCGACA	AAATCATTTT	ATTATTTTTG	CTAATGATTC	TAATCAAAAT 24
O. harrisiana 27				A				
O. ridleyana 61				A				
O. ridleyana 56 Ophiorrhiza sp. 35		•••••		A				
O. pumila								
O. kuroiwae O. pseudofasciculata 37				A				
O. ridleyana 52				A				
O. pedunculata 41								
O. trichocarpon 46 O. iaponica		••••		A				
O. hayatana				A				24
				A				AC
O. plumbea 6	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	А 280 ССТСАААССА	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	AC24 320 TTCCATTTGA 32
O. plumbea 6 O. harrisiana 18 O. harrisiana 27	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	ССТСАААССА	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	AC24 320 I TTCCATTTGA 32 32
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. diucosa 64	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	ССТСАААССА	TATCAGAAGG	300 GTTTGCATTT	ATTGTGGAAA	AC
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 50	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	300 GTTTGCATTT	ATTGTGGAAA	AC
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	300 GTTTGCATTT	ATTGTGGAAA	AC
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C24 300 TTCCATTTGA 32 32 32 32 32 32 32 32 32 32 32 32 32 3
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C24 300 TTCCATTTGA 32 32 32 32 32 32 32 32 32 32 32 32 32 3
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C24 330 TTCCATTTGA 22 23 23 23 23 23 23 23 23 23 23 23 23 2
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C24 330 TTCCATTTGA 22 23 23 23 23 23 23 23 23 23 23 23 23 2
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C24 300 TTCCATTTGA 22 23 23 23 23 23 23 23 23 23
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	CGAGTTTTTG	C. T. 340	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 2 23 23 23 23 23 23 23 23 23 2
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6	CGAGTTTTTG	C.T.	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	ATTTACGATC	ATTGTGGAAA	A.C24 300 TTCCATTTGA 2 32 32 32 32 32 32 32 32 32 3
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, parrisiana 27	CGAGTTTTTG	C.T.	GAATTTCTAT	T. TG AAGGGTATTC	AAATCTCATA	A. 300 ATTTACGATC	ATTGTGGAAA	A.C24 300 TTCCATTTGA 22 320 322 323 323 323 323 323 3
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64	CGAGTTTTTG	C.T.	GAATTTCTAT	TTG AAGGGTATTC AAGAGGTATTC	AAATCTCATA	A	ATTGTGGAAA	A.C24 300 TTCCATTTGA 22 320 322 323 323 323 323 323 3
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucleyana 61 O, ridleyana 56	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	TTG. AAGGGTATTC AAGGGTATTC AAGA	AAATCTCATA	A	ATTGTGGAAA	A.C24 300 TTCCATTTGA 22 320 322 323 323 323 324 325 325 325 325 327 327 327 327 327 327 327 327
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucleyana 56 O, ridleyana 56 O, ridleyana 56 Ophiorrhiza sp. 35	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	TTG. AAGGGTATTC AAGGGTATTC AAGA	AAATCTCATA	A	ATTGTGGAAA	A.C
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	CCTCAAACCA	AAATCTCATA	A	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 22 320 320 322 322 322 322 322 3
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 17 O, fucosa 64 O, irdleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pumila O, pumila O, pumila O, pumila O, pumila O, purdeana 52	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	TTG. 300 AAGGGTATTC AAGGGTATTC AAAAAAAAAAAAAAAAA	AAATCTCATA	A A ATTTACGATC	ATTGTGGAAA	A.C
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, irdleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	TTG. 300 AAGGGTATTC AAGGGTATTC AAGA AA AA AA AA AA AA AA AA	AAATCTCATA	A A ATTTACGATC	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 32 32 32 32 32 32 32 32 32 32
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, hayratana Joosia umbellifera O, plumbea 6 O, harrisiana 17 O, fucosa 64 O, irdleyana 52 O, pumbea 6 O, harrisiana 27 O, fucosa 64 O, irdleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, seeudofasciculata 37 O, ridleyana 52 O, pumila O, seudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pumila O, seudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	TTG. 300 AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA A	AAATCTCATA	A A ATTTACGATC	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 22 320 320 322 322 322 322 322 3
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 66 Ophiorrhiza sp. 35 O, purnila O, seduofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, harrisiana 18 O, harrisiana 27 O fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, pedunculata 41 O, richocarpon 46 O, japonica	CGAGTTTTTG	GTTGCAACAA	GAATTTCTAT	TTG. 300 AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA A	AAATCTCATA	A A ATTTACGATC	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 22 320 320 322 322 322 322 322 3
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, purnila O, ridleyana 50 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, huroisaa 18 O, harrisiana 17 O, fucosa 64 O, ridleyana 61 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, pedunculata 41 O, trichocarpon 46 O, japonica	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT	TTG. 300 AAGGGTATTC AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA A	TATCAGAAGG	A A ATTTACGATC	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 32 32 32 32 32 32 32 32 32 32
Ocusia a antecimida O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pedunculata 41 O. trichocarpon 46 O. japonica O. harrisiana 17 O. fucosa 64 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 61 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT	TTG. 300 AAGGGTATTC AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA A		A A ATTTACGATC	ATTGTGGAAA	A. C
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophioritza sp. 35 O. purnila O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 61 O. ridleyana 61 O. ridleyana 52 O. pseudofasciculata 62 O. pseudofasciculata 62 O. pseudofasciculata 62 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana O. kuroiwae O. pseudofasciculata 62 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT	TTG. 300 AAGGGTATTC AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA A	ТАТСАGAAGG	A A ATTTACGATC	ATTGTGGAAA	A. C
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophioritza sp. 35 O, purnila O, ridleyana 50 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 52 O, pseudofasciculata 62 O, purnila O, ridleyana 61 O, ridleyana 61 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, burniwae O, purnila O, seudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT	TTG. 300 AAGGGTATTC AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA A	TATCAGAAGG	A A ATTTACGATC	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 22 320 321 322 322 322 322 322 322 322
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, purnila O, ridleyana 50 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, japonica O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 61 O, ridleyana 61 O, ridleyana 63 Ophiorrhiza sp. 35 O, purnila O, seudofasciculata 37 O, ridleyana 63 Ophiorrhiza sp. 35 O, purnila O, seudofasciculata 37 O, ridleyana 56 Ophiorrhiza sp. 35 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT	TTG. 3000 AAGGGTATTC AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA A	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 32 32 32 32 32 32 32 32 32 32
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, purulia O, ridleyana 50 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, paedunculata 41 O, trichocarpon 46 O, japonica O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 61 O, ridleyana 63 O, purulia O, seudofasciculata 37 O, ridleyana 63 O, purulia O, seudofasciculata 37 O, ridleyana 50 O, pagonica O, pagonica O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35	CGAGTTTTTG	2000 GTTGCAACAA 	GAATTTCTAT	TTG AAGGGTATTC AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA A	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 22 320 TTCCATTTGA 22 32 32 32 32 32 32 32 32 32
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, japonica O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, nidleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pedunculata 41 O, tridleyana 56 Ophiorrhiza sp. 35 O, pseudofasciculata 62 O, pedunculata 41 O, tridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, tridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, tridleyana 50 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 61 O, ridleyana 61 O, pumila O, pumila	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 22 320 TTCCATTTGA 22 32 32 32 32 32 32 32 32 32
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, japonica O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, iridleyana 50 O, pumila O, kuroiwae O, pedunculata 41 O, ridleyana 50 O, pumila O, kuroiwae O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT	CCTCAAACCA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 22 320 TTCCATTTGA 22 32 32 32 32 32 32 32 32 32
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, hapatana Joosia umbellifera O, plumbea 6 O, harrisiana 27 O, fucosa 64 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 50 O, pumila O, kuroiwae O, pedunculata 41 O, trichocarpon 46 O, japonica O, payatana Joosia umbellifera O, pedunculata 62 O, pedunculata 62 O, pumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 50 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 50 O, pumila	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT	CCTCAAACCA CCTCAAACCA TTG. AAGGGTATTC AAGGGTATTC AA AA AA AA AA AA AA AA AA AA AA AA AA	TATCAGAAGG	GTTTGCATTT	ATTGTGGAAA	A.C
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae C, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, japonica O, harrisiana 27 O, fucosa 64 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, ridleyana 50 O, pumila O, kuroiwae O, pedunculata 41 O, ridleyana 50 O, pumila O, kuroiwae O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pounbea 6 O, harrisiana 18 O, harrisiana 17 O, fucosa 64 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 50 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 62 O, pedunculata 41 O, tridheyana 52 O, pumila	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT AG AAGAGGGGAA	CCTCAAACCA CCTCAAACCA TTG. AAGGGTATTC AAAAAAAAAA.	TATCAGAAGG	A A ATTTACGATC	ATTGTGGAAA	A. C
O, plumbea 6 O, harrisiana 17 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae C, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 27 O, fucosa 64 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 51 O, ridleyana 50 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera D, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pagyatana Joosia umbellifera C, hayatana Joosia umbellifera O, harrisiana 18 O, harrisiana 17 O, fucosa 64 O, pumila 0, kuroiwae O, pseudofasciculata 37 O, o, japonica O, japonica	CGAGTTTTTG	200 GTTGCAACAA 	GAATTTCTAT AG AAGAGGGGAA	CCTCAAACCA CCTCAAACCA TTG. AAGGGTATTC AAAAAAAAAA.	TATCAGAAGG	A A ATTTACGATC	ATTGTGGAAA	A.C. 24 300 TTCCATTTGA 22 320 322 322 323 323 323 323 3

		600		600		540		600	
		1		1				1	
O. plumbea 6 O. barrisiana 18	CAAACCCTTC	GTTATTGGGT	AAAAGATGCC	TCTTCCTTGC	ATTTATTACG	ATTCTTTTTC	CACAAGTATT	GGAGTTGGAA	560
O harrisiana 27									560
O. fucosa 64									560
O. ridleyana 61									560
O. ridleyana 56				• • • • • • • • • • •					560
O pumila									560
O. kuroiwae									560
O. pseudofasciculata 37									560
O. ridleyana 52				• • • • • • • • • • •					560
O. pedunculata 41	с								560
O. trichocarpon 46									560
O. japonica									560
O. nayatana									560
Joosia uniberniera	A			C			6	A	560
		1		1		1			
O. plumbea 6	TACTCTTATT	GCTACAAGCA	AACCCTGTTT	GGATTTTTCA	CCAAAAAGAA	ATCAAAGATT	GTTTTTCTTA	TTATATAATT	640
O harrisiana 27									640
O. fucosa 64		G .							640
O. ridleyana 61	<mark>G</mark> .	G .							640
Onbiorrhiza sp. 35	<mark>G</mark> .	G.							640
O, pumila									640
O. kuroiwae		G .							640
O. pseudofasciculata 37		AG.		G					640
O. ridieyana 52		AG.							640
O. pedunculata 41		AG.							040 640
O. trichocarpon 46									640
O. japonica		AG.							640
U. nayalana Joosia umbellifera		AG.							640
		AAG.	····· A · · · ·			3.3	AAC	720	040
				ĩ		1		Ĩ	
O piumpea 6 O barrisiana 18	CACATGTATA	TGAATACGAA	TCCATTTTTG	CCTTTCTCCG	TAAGCAATCT	TCTCATTIGG	GATCAACATC	TTTTGGAGTC	720
O. harrisiana 27									720
O. fucosa 64									720
O. ridleyana 61									720
O. ridleyana 56 Ophiorrhizo op. 35		•••••							720
Ophionniza sp. 55 O pumila					C				720
O. kuroiwae									720
O. pseudofasciculata 37		• • • • • • • • • • •	G.						720
O. ridleyana 52		•••••	G.						720
O. pseudolasciculata 62			G.						720
O. trichocarpon 46			G.						720
O. japonica		. <mark></mark> <mark></mark>	G.						720
O. hayatana			T.G.						700
loosia limbellitera	тс			T T					720
Joosia umbellifera	. T G		c.	T	c	C			720
Joosia umbellifera	. T G	740 1	c.	T T 760	c	C 780		800 I	720
Joosia umbellifera O. plumbea 6 O. barrisiana 18	.TG	GAATATATTT	CTACGGAAAA	TT 760 AAAGAAAGGC	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG		720 720 800
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27	TTTCTTGAAC	740 I GAATATATTT	CTACGGAAAA	TT 760 AAAGAAAGGC	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	800 I TTAGTTTATG	720 720 800 800 800
Joosia umbellitera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64	TTTCTTGAAC	740 I GAATATATTT	CTACGGAAAA	TT 760 AAAGAAAGGC	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	TTAGTTTATG	720 720 800 800 800 800
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61	.TG	740 I GAATATATTT	CTACGGAAAA	TT AAAGAAAGGC	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	800 I TTAGTTTATG	720 720 800 800 800 800 800
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 53	.TG	740 GAATATATTT	CTACGGAAAA	TT 780 AAAGAAAGGC	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	TTAGTTTATG	720 720 800 800 800 800 800 800 800
Joosia umbeilifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila	.TG	740 GAATATATTT	CTACGGAAAA	TT 760 AAAGAAAAGGC 	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	800 I TTAGTTTATG	720 720 800 800 800 800 800 800 800 800 800
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae	.TG	GAATATATTT	CTACGGAAAA	TT 780 AAAGAAAGGC G.	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	800 TTAGTTTATG	720 720 800 800 800 800 800 800 800 800 800
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37	.TG	GAATATATTT	CTACGGAAAA	TT 760 AAAGAAAGGC	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	TTAGTTTATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ordleyana 52 O. pseudofasciculata 52	.TG	GAATATATTT	CTACGGAAAA	TT 780 AAAGAAAGGC G	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	BOO I TTAGTTTATG	720 720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41	.TG	GAATATATTT	CTACGGAAAA	TT 780 AAAGAAAGGC 	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	BOO I TAGTITATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwaa O. seudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46	.TG	GAATATATTT	CTACGGAAAA	TT PROVIDE TO THE TOTAL STREET	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	TTAGTTTATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica	.TG	GAATATATTT	CTACGGAAAA	TT Prevention of the second secon	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	BOO I TTAGTTTATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Jonsia umbellifera	.TG	GAATATATTT	CTACGGAAAA	TT 700 AAAGAAAGGC 	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	BOO TTAGTTTATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbeilifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbeilifera	.TG	240 I GAATATATTT	CTACGGAAAA	TT 7760 AAAGAAAGGC 	TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	B800	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwaa 7 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera	T G		CTACGGAAAA	TT 7760 1 AAAGAAAGGC 	C	CGTTGCGGAG	GATTTTCAGG		720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwaa O. pseudofasciculata 37 O. ridleyana 52 O. psedudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18	T G	GAATATATTT GAATATATTT GACCTTTCA GACCCTTTCA GCCCTTTCA	CTACGGAAAA	TT 7700 AAAGAAAGGC G. G. G. 	C. TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	BBO TTAGTTTATG CCTCTTTTGA	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 27	T G	GAATATATTT GAATATATTT	CTACGGAAAA	TT 7760 1 AAAGAAAGGC 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	BOO TTAGTITATG 	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbeilifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 6 O. jaurea 24	T G	GAATATATTT GAATATATTT GACCCTTTCA GACCCTTTCA G	CTACGGAAAA	TT 770 AAAGAAAGGC G. G. 	C. TTGTAGAAGT	TTCTGGTTTC	GATTTTCAGG	B80 ITAGTITATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwaa 7 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayratana Joosia umbellifera O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. d. ridleyana 61 O. ridleyana 56	T G	GAATATATTT GAATATATTT GACCCTTTCA GACCCTTTCA GGCCCTTCA GGCCCTTCA GGCCCTTCA	CTACGGAAAA	TT 7960 AAAGAAAGGC 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	BBO I TTAGTTTATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. psedunculata 42 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. harrisiana 18 O. harrisiana 27 O. fudeyana 61 O. ridleyana 61 O. ridleyana 61 O. ridleyana 53	TTTCTTGAAC	CAATATATTT GAATATATTT Bandaria GAACCCTTTCA GACCCTTTCA GG GG GG GG GG GG GG GG GG GG GG GG GG	CTACGGAAAA	TT 7700 AAAGAAAGGC G. G. 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	TTAGTITATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 67 O. ridleyana 52 O. pseudofasciculata 41 O. ridleyana 52 O. pseudofasciculata 41 O. japonica O. japonica O. japonica O. hayatana Joosia umbellifera O. harrisiana 18 O. harrisiana 17 O. ridleyana 61 O. ridleyana 61 O. ridleyana 52 O. pidenana 17 O. ridleyana 53 O. pumila	T G	CAATATATTT GAATATATTT GAATATATTT Same series of the series	CTACGGAAAA	TT 7700 P AAAGAAAGGC G G C C C T C T C T C T C T C T C T C T C 	GGAAAATCAA	TTCTGGTTTC	GATTTTCAGG	CCTCTTTTGA	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. ridleyana 52 O. pseudofasciculata 41 O. japonica O. jayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. plumila	TTTCTTGAAC	GAATATATTT GAATATATTT GACCCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 77800 I AAAGAAAGGC G G C C TCTT. TAGGTATCAA	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	BOO ITAGTITATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwaa O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayratana Joosia umbellifera O. harrisiana 18 O. harrisiana 27 O. fudeyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37	T G	GAATATATTT GAATATATTT GACCTTTCA GACCCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 7700 AAAGAAAGGC G. G. 	C. TTGTAGAAGT	TTCTGGTTTC	GATTTTCAGG	BBO TTAGTTTATG	720 720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwaa O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayratana Joosia umbellifera O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae	T G	CAATATATTT GAATATATTT Band State GAACCCTTTCA GAACCCTTTCA GG GG GG GG GG GG GG GG GG GG GG GG GG	CTACGGAAAA	TT 7700 AAAGAAAGGC G. G. 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	BOO TTAGTITATG CCTCTTTTGA	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. poumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. ridleyana 61 O. ridleyana 66 Ophiorrhiza sp. 35 O. pseudofasciculata 37 O. ridleyana 61 O. kuroiwae O. pseudofasciculata 37 O. pseudofasciculata 37 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37	TTTCTTGAAC	GAATATATTT GAATATATTT GACCCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 7700 AAAGAAAGGC G. G. C C TCTT. B40 TAGGTATCAA	GGAAAATCAA	TTCTGGTTTC	GATTTTCAGG	B00 TTAGTITATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayratana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 17 O. ridleyana 61 O. ridleyana 66 Ophiorrhiza sp. 35 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46	TTTCTTGAAC	GAATATATTT GAATATATTT GACCCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 77800 I AAAGAAAGGC G G 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	BOO ITAGTITATG	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 97 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayriana 27 O. fucosa 64 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. plumbea 6 O. harrisiana 18 O. polumbea 76 O. pideyana 52 O. pumila O. ridleyana 52 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 32 O. pseudofasciculata 32 O. pedunculata 41 O. trichocarpon 46 O. japonica O. havatana	T G	GAATATATTT GAATATATTT GACCCTTTCA GACCCTTTCA GACCCTTTCA GG GG GG GG GG GG GG GG GG GG GG GG GG	CTACGGAAAA	TT 7960 AAAGAAAGGC G. G. 	C. TTGTAGAAGT	TTCTGGTTTC	GATTTTCAGG	BBO TTAGTTTATG CCTCTTTTGA	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 44 O. japonica O. harrisiana 27 O. fucosa 64 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 61 O. ridleyana 61 O. pumila O. pedunculata 41 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 62 O. pedunculata 64 O. japonica O. japonica O. hayatana Joosia umbellifera	.T	CAATATATTT GAATATATTT GAACCTTTCA GACCCTTTCA GG GG GG GG GG GG GG GG GG GG GG GG GG	CTACGGAAAA	TT 7760 AAAGAAAGGC G. G. 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	BOO TTAGTITATG CCTCTTTTGA	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. trichocarpon 46 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. ridleyana 61 O. ridleyana 61 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 56 Ophiorrhiza sp. 35 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37	T G	GAATATATTT GAATATATTT GAATATATTT GACCCTTTCA GGCCCTTTCA GGCCG GGCGGG GGCGG GGGGG GGGGGG	CTACGGAAAA	TT 7760 1 AAAGAAAGGC 	GGAAAATCAA	TT. TA.	GATTTTCAGG	BOO TTAGTITATG CCTCTTTTGA CCTCTTTTGA	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. trichocarpon 46 O. japonica O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. ridleyana 65 Ophiorrhiza sp. 35 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera	.T	GAATATATTT GAATATATTT GAACCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 7700 AAAGAAAGGC GG. CCTT. TAGGTATCAA TAGGTATCAA	GGAAAATCAA	TT. TA. BEORE	GATTTTCAGG	CCTCTTTTGA	720 720 800 800 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 97 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayriana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. pridleyana 52 O. pumbea 6 O. pidenya 52 O. pseudofasciculata 32 O. pumbea 6 O. huroiwae O. pseudofasciculata 62 O. pedunculata 41 O. ridleyana 52 O. pumila O. pumila O. sulowae O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayratana Joosia umbellifera	.TG TTTCTTGAAC 	GAATATATTT GAATATATTT GAACCTTTCA GACCCTTTCA GGACCCTTCA GGAAATCTTAT	CTACGGAAAA	TT 7760 AAAGAAAGGC G. G. 	C. TTGTAGAAGT	TT. TA Below TTCTGGTTTC	GATTTTCAGG AAAGGATACG AAAGGATACG AAGG ACTCGGGAAG	BBO TTAGTITATG CCTCTTTTGA CCTCTTTTGA CCTCTTTTGA CCTCTTTTGA GTTCTATATA	720 720 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 17 O. fucosa 64 O. ridleyana 61 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. trichocarpon 46 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 17 O. ridleyana 52 O. pseudofasciculata 17 O. ridleyana 52 O. pseudofasciculata 17 O. ridleyana 54 O. padunculata 41 O. trichocarpon 46 O. japonica O. harrisiana 18 O. harrisiana 27	T G TTTCTTGAAC 	GAATATATTT GAATATATTT GAACCCTTTCA GACCCTTTCA GG GG GG GG GG GG GG GG GG GG GG GG GG	CTACGGAAAA	TT 7760 AAAGAAAGGC G. T. C. T. CTT. TAGGTATCAA TAGGTATCAA 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	CC.	720 720 800 800 800 800 800 800 800 8
Joosia umbeliifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. kuroiwae O. pseudofasciculata 37 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. polumbea 6 O. harrisiana 17 O. fucosa 64 O. harrisiana 18 O. harrisiana 17 O. plumbea 6 O. harrisiana 17 O. fucosa 64 O. piumbea 6 O. harrisiana 17 O. ridleyana 52 O. plumbea 6 O. harrisiana 17 O. ridleyana 27 O. fucosa 64 O. harrisiana 17 O. ridleyana 27 O. ridleyana 27 O. plumbea 6 O. harrisiana 17 O. ridleyana 27 O. ridleyana 27 O. fucosa 64 O. harrisiana 17 O. ridleyana 27 O.	T G	GAATATATTT GAATATATTT GAATATATTT GAACCCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 77800 1 AAAGAAAGGC 	GGAAAATCAA	TT. TA.	GATTTTCAGG	BOO TTAGTITATG CCCCCTTTTGA CCCCCTTTTGA CCCCCTTTTGA CCCCCCCCCC	720 720 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. hayatana Joosia umbellifera	.T	GAATATATTT GAATATATTT GACCCTTTCA GACCCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 7700 AAAGAAAGGC G	GGAAAATCAA	TTCTGGTTTC	GATTTTCAGG	BBO TTAGTITATG CCCCCTTTTGA CCCCCTTTTGA CCCCCCCCCCC	7200 7200 8000 8000 8000 8000 8000 8000
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 97 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayriana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 52 O. pseudofasciculata 32 O. pumila O. kuroiwae O. pseudofasciculata 62 O. pedunculata 41 O. ridleyana 56 Ophiorrhiza sp. 35 O. japonica O. hayrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35	.TG TTTCTTGAAC 	GAATATATTT GAATATATTT GAACCCTTTCA GACCCTTTCA GG GG GG GG GG GG GG GAAATCTTAT GAAATCTTAT	CTACGGAAAA	TT 7700 AAAGAAAGGC G. 	C. TTGTAGAAGT	CGTTGCGGAG	GATTTTCAGG	BOO TTAGTITATG CCTCTTTTGA CCTCTTTTGA CCTCTTTTGA GTTCTATATA	720 720 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 17 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. ridleyana 52 O. pseudofasciculata 41 O. ridleyana 61 O. harrisiana 17 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 72 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. palmbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. ridleyana 61 O. ridleyana 61 O. ridleyana 61 O. pumila	.T	GAATATATTT GAATATATTT GAATATATTT GAACCCTTTCA GG GG GG GG GG GG GG GG GG GG GG GG GG	CTACGGAAAA	TT 7760 AAAGAAAGGC 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	CCCCCTTTTGA	720 720 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 55 O. pumila O. plumbea 6 O. harrisiana 27 O. ridleyana 61 O. ridleyana 55 O. pumila O. kuroiwae O. pseudofasciculata 41 O. trichocarpon 46 O. harrisiana 27 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 50 O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 50 O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 50 O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 50 O. plumbea 6 O. plumbea 6 O. plumbea 6 O. plumbea 6 O. plumbea 7 O. fucosa 64 O. ridleyana 50 O. plumbea 7 O. fucosa 64 O.	T G	GAATATATTT GAATATATTT GAACCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 7700 AAAGAAAGGC GG. T.C.T. TAGGTATCAA	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG	CCTCTTTTGA	720 720 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pula O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. hayriana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 52 O. pseudofasciculata 37 O. fucosa 64 O. harrisiana 18 O. harrisiana 27 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. payatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 65 Ophiorrhiza sp. 35 O. pumila O. pumila O. pumila O. ridleyana 53 O. pumila O. pumila O. sucosa 64 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 52 O. pumila O. pumila	.T	GAATATATTT GAATATATTT GAACCCTTTCA GACCCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 7960 AAAGAAAGGC 	GGAAAATCAA	TTCTGGTTTC	GATTTTCAGG	BBO TTAGTITATG CCCCTTTTGA CCCCCTTTTGA CCCCCTTTTGA GTTCTATATA	720 720 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 53 O. pumila O. pedunculata 41 O. trichocarpon 46 O. japonica O. japonica O. pagyana 52 O. pagyana 52 O. pumila O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 50 O. pumila O. kuroiwae	.TG TTTCTTGAAC 	GAATATATTT GAATATATTT GAACCTTTCA GACCCTTTCA GG GG GG GG GG GG GG GAAATCTTAT GAAATCTTAT	CTACGGAAAA	TT 7760 AAAGAAAGGC G. 	GGAAAATCAA	CGTTGCGGAG	GATTTTCAGG AAAGGATACG AAAGGATACG AAAGGATACG ACTCGGGAAG A A A A A A A A A A A A A A	CCTCTTTTGA	720 720 800 800 800 800 800 800 800 8
Joosia umbellifera O. plumbea 6 O. harrisiana 17 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. piumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. trichocarpon 46 O. harrisiana 18 O. harrisiana 27 O. ridleyana 61 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumbea 6 O. harrisiana 18 O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 16 O. harrisiana 18 O. harrisiana 17 O. ridleyana 61 O. ridleyana 61 O. ridleyana 52 O. pumila O. pumila O. kuroiwae O. pumila	.T	GAATATATTT GAATATATTT GAATATATTT GACCCTTTCA GGCCCTTTCA GGCCCG GGCG GG	CTACGGAAAA	TT 7700 AAAGAAAGGC G. 	GGAAAATCAA	TT. TA.	GATTTTCAGG	CCCCCTTTTGA	720 720 8000 8000 8000 8000 8000 8000 80
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. harrisiana 18 O. haryatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 65 Ophiorrhiza sp. 35 O. pseudofasciculata 37 O. ridleyana 61 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. harrisiana 18 O. harrisiana 17 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. ridleyana 52 O. pseudofasciculata 41 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. kuroiwae O. pseudofasciculata 41 O. ridleyana 52 O. pseudofasciculata 41 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica	.T	GAATATATTT GAATATATTT GAACCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 7700 AAAGAAAGGC GG. T.CTT TAGGTATCAA	GGAAAATCAA	TT. TA. BEORE	GATTTTCAGG AAAGGATACG AAAGGATACG AAAGGATACG ACTCGGGAAG A A A A A A A A A	CCTCTTTTGA	720 720 8000 800 800 800 800 800 800 800 800
Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. padunculata 41 O. trichocarpon 46 O. japonica O. plumbea 6 O. harrisiana 18 O. plumbea 6 O. harrisiana 12 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pedunculata 41 O. trichocarpon 46 O. japonica	.T	GAATATATTT GAATATATTT GACCCTTTCA GACCCTTTCA GACCCTTTCA G G G G G G G G G G G G G G G G G G G	CTACGGAAAA	TT 7700 AAAGAAAGGC 	GGAAAATCAA	TTCTGGTTTC	GATTTTCAGG	CCTCTTTTGA	720 720 80000 8000 8000 8000 8000 8000 8000 8000 8000 8000 8

		980		1,000		1,020)	1,040	
O skymskaa G				A TOCOCTA TO	****			TACCCACCO	1010
O. plumbea 6	AAIGAAITAI	CLAATCATTC	CITIGACITI	AIGGGCIAIC	TTTCAAGIGI	GCAACTAAGC	CUGICAAIGG	TACGGAGCCA	1040
O. harrisiana 10									1040
									1040
O. ridlovono 61									1040
O. ridlovana 56									1040
Onbiorrhiza en 35						Δ			1040
O pumila						Δ			1040
O kuroiwae									1040
O nseudofasciculata 37									1040
O ridlevana 52					T	Δ			1040
O nseudofasciculata 62						Δ			1040
O nedunculata 41						Δ			1040
O trichocarpon 46						A .			1040
O iaponica						A .	.T		1040
O havatana						A .			1040
Joosia umbellifera		<mark>C</mark>	. C A	A T T		G A .	T	A T	1040
oooola ambomora		1.060)	1.080		1.100)	1,120	
		1		1		1		1	
O. plumbea 6	AATGCTAGAA	AATTCATTTC	TAATCAATAA	TGCTATTAAG	AAATTGGATA	CCCTTGTTCC	AATTATTCCT	CTTATTGGAT	1120
O. harrisiana 18									1120
O. narrisiana 21									1120
O. Iucosa 64									1120
O. ridleyana 61			• • • • • • • • • • •						1120
O. ridieyaria 56			• • • • • • • • • •						1120
Opniormiza sp. 35				• • • • • • • • • • •					1120
O. punna									1120
O providefensionlate 37									1120
O ridlevana 52									1120
O nseudofasciculata 62		6							1120
O nedunculata 41		6							1120
O trichocarpon 46		6			T				1120
O ianonica					T				1120
O havatana					T				1120
Joosia umbellifera		G						· · · · · · · · · · · · · · · · · · ·	1120
boosia ambennera				4.480		4.40/		1 200	1120
		1,14		1,100		1,100	,	1,200	
O. plumbea 6	CATTGGCTAA	AGCGCAATTT	TGTAACCTAT	TAGGACATCC	CGTTAGTAAG	CCGGTTTGGG	CTGATTTATC	AGATTCTGAT	1200
O. harrisiana 18						<mark>A</mark>			1200
O. harrisiana 27						<mark>A</mark>			1200
O. fucosa 64	<mark>.</mark>					A			1200
O. ridleyana 61	<mark></mark>					<mark>A</mark>			1200
O. ridleyana 56						<mark>A</mark>			1200
Ophiorrhiza sp. 35						<mark>A</mark>			1200
O. pumila						<mark>A</mark>			1200
O. kuroiwae									1200
O. pseudofasciculata 37					G				1200
O. ridleyana 52		• • • • • • • • • • •			G				1200
O. pseudofasciculata 62					G				1200
O. pedunculata 41									1200
O. trichocarpon 46									1200
O. japonica							. C		1200
O. hayatana		. 							1200
Joosia umbellitera									1200
		CA				A			1200
		CA		1.240		A 1,260	,	1,280	1200
O plumbes 6	ATTATTGACC	CA 1.220 1 CATTIGGGTA	TATATGCAGA	1,240	ATTATCATAG	A 1,260		1,280 I CTTTCTATCC	1200
O. plumbea 6 O. harrisiana 18	ATTATTGACC	GATTTGGGTA	TATATGCAGA	1.240 AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAA	1.280 I GTTTGTATCG	1200 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAGA	1.280 I GTTTGTATCG	1200 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O fucosa 64	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAA	1.280 I GTTTGTATCG	1200 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. didevana 61	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAA	1.280 GTTTGTATCG	1200 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. dideyana 56	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAGA	1.280 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza so. 35	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAGA	1.280 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAGA	1.280 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAGA	1.200 I GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. purnila O. kuroiwae O. pseudofasciculata 37	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAA	GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAGA	GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. pseudofasciculata 62	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAA	GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 32 O. pseudofasciculata 34	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAA	GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwaa 7 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, psedunculata 41 O, trichocarpon 46	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAA	GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 64	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAA	GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 32 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAA	GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroïwaa O. seudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAGA	gtttgtatcg	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAA 	1.200 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	ATTATTGACC	GATTTGGGTA		1.244 AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAA	1.280 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, turoiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, paponica O, hayatana Joosia umbellifera O, plumbea 6 O, partisiana 18	ATTATTGACC C	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAGA	1.200 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18	ATTATTGACC .C.	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAA 	1.200 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64	ATTATTGACC	GATTTGGGTA	TATATGCAGA	1.246 AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAGA	1.200 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61	ATTATTGACC C	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAGA	1.200 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	T. A. 1300 T. A. 1300 T. A. 1300 T. A. 1300 T. A. 1300 T. A. 1300 T. 13000 T. 1300 T. 13000 T. 13000 T. 13000 T. 1300	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1.280 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 62 O. paedunculata 62 O. paedunculata 62 O. paedunculata 64 O. japonica O. hayatana Joosia umbellifera O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 61 O. ridleyana 61 O. ridleyana 50	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC 	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAGA	1.200 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 17 O, fucosa 64 O, hardeyana 56 Ophiorrhiza sp. 35 O, pumla	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	GTTTGTATCG	1200 1200 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 66 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAGA	1.200 GTTTGTATCG	1200 1200 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 61 O. ridleyana 61 O. ridleyana 61 O. ridleyana 53 O. pumila O. kuroiwae O. pumila O. kuroiwae O. pseudofasciculata 37	ATTATTGACC C	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAGA	1.200 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 31 O, iqoponica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 55 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	GCTCGGAAAC	CGGTTCTTCC	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1,280 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, purnila O, seudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 61 O, fucosa 61 O, pumila O, kuroiwae O, pseudofasciculata 37 O, pseudofasciculata 62	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC AACCTTTCTC	ATTATCATAG	T.A. ACAAAAGTAC	AAAAAAAAAAAAGA	1.200 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, rideyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, psedunculata 41 O, trichocarpon 46 O, japonica O, harrisiana 18 O, harrisiana 18 O, harrisiana 17 O, ridleyana 61 O, ridleyana 61 O, ridleyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAGA	1.200 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 56 Ophiorrhiza sp. 35 O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. ridleyana 52 O. pumila O. kuroiwaa 75 O. pumila O. kuroiwaa 75 O. ridleyana 52 O. pseudofasciculata 37 O. ridleyana 52 O. pumila O. kuroiwaa 75 O. ridleyana 52 O. pseudofasciculata 31 O. ridleyana 52	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAGA 	1,200 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 Ophiorrhiza sp. 35 O. purnila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 41 O. trichocarpon 46 O. japonica O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. harrisiana 27 O. fucosa 61 O. ridleyana 61 O. ridleyana 61 O. pumila O. kuroiwae O. pumila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. pedunculata 61 O. pedunculata 62 O. pedunculata 62	ATTATTGACC CA. AATAAAGTAT	GATTTGGGTA	TATATGCAGA	AACCTTTCTC AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAGA	1.200 GTTTGTATCG	1200 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisana 27 O, ridleyana 55 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, japonica	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	T. A	AAAAAAAAAAAA G TGTACGTGTT	1,280 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, purnila O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 55 O, pumila O, seudofasciculata 62 O, pedunculata 41 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, hayatana Joosia umbellifera	ATTATTGACC .C.	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	T.A. A A 1.200 1.20	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1,200 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae C, pseudofasciculata 37 O, ridleyana 52 O, pseduofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera C, plumbea 6 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 62 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 44 O, japonica O, hayatana Joosia umbellifera	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAA G TGTACGTGTT	1,220 GTTTGTATCG	1280 1280 1280 1280 1280 1280 1280 1280
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, pumbea 6 Ophiorrhiza sp. 35 O, pumila O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 32 O, pedunculata 41 O, ridleyana 52 O, pseudofasciculata 32 O, pedunculata 42 O, pedunculata 41 O, ridleyana 50 O, japonica O, japonica O, hayatana Joosia umbellifera	ATTATTGACC	GATTTGGGTA		AACCTTTCTC AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAA 	1,200 GTTTGTATCG	12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 136000 136000 136000 1360000000000
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, purnila O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, paedunculata 41 O, trichocarpon 46 O, japonica O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, barrisiana 37 O, ridleyana 56 Ophiorrhiza sp. 35 O, pseudofasciculata 37 O, ridleyana 50 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	ATTATTGACC CA. AATAAAGTAT	GATTTGGGTA	TATATGCAGA	AACCTTTCTC AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAGA 	1,200 GTTTGTATCG 1,300 TTTTTGAAAA GGCTTCTTCG	12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 136000 136000 136000 1360000000000
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, rideyana 61 O, rideyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, rideyana 55 O, pumila O, kuroiwae O, pseudofasciculata 35 O, pideyana 55 O, pumila O, kuroiwae O, pseudofasciculata 35 O, pideyana 52 O, pseudofasciculata 35 O, rideyana 52 O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC AACCTTTCTC	ATTATCATAG GCTCGGAAAC 	CGGTTCTTCC	AAAAAAAAAAAAA G TGTACGTGTT G G G G G G C C ACTTCCCAAG	GTTTGTATCG	12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 136000 136000 136000 1360000000000
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, purnila O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 17 O, ridleyana 61 O, ridleyana 61 O, ridleyana 61 O, ridleyana 61 O, purnila O, purnila O, purnila O, seudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	ATTATTGACC CA. AATAAAGTAT	GATTTGGGTA	TATATGCAGA	AACCTTTCTC AACCTTTCTC	ATTATCATAG	СGGTTCTTCC 	AAAAAAAAAAAA G TGTACGTGTT G G G G G G G G C ACTTCCCAAG	1,200 GTTTGTATCG 1,300 TTTTTGAAAA GGCTTCTTCG	12800 13600 136000 13600 136000 136000 10000000000
O, plumbea 6 O, harrisiana 17 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae C, pseudofasciculata 37 O, ridleyana 52 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 51 O, pedunculata 41 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, fucosa 64 O, harrisiana 18 O, harrisiana 17 O, ridueyana 61	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAA G TGTACGTGTT G G G G G C ACTTCCCAAG	1,220 GTTTGTATCG	12800 13600 136000 13600 13600 136000 136000 10000000000
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, plumbea 6 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, nidleyana 56 Ophiorrhiza sp. 35 O, pumila O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, ridleyana 52 O, pedunculata 41 O, ridleyana 52 O, pedunculata 41 O, ridleyana 52 O, pedunculata 41 O, irdleyana 52 O, pedunculata 41 O, hayatana Joosia umbellifera	ATTATTGACC C	GATTTGGGTA	TATATGCAGA A TTTCTTGTGT	AACCTTTCTC AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAGA 	1,200 GTTTGTATCG	12800 13600 136000 13600 136000 136000 10000000000
O. plumbea 6 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 61 O. ridleyana 66 Ophioritza sp. 35 O. purnila O. kuroiwae O. pseudofasciculata 37 O. ridleyana 52 O. pseudofasciculata 62 O. paedunculata 41 O. trichocarpon 46 O. japonica O. hayatana Joosia umbellifera O. plumbea 6 O. harrisiana 27 O. fucosa 64 O. harrisiana 18 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. harrisiana 27 O. fucosa 64 O. piaphaea 61 O. ridleyana 61 O. ridleyana 50 S. pseudofasciculata 37 O. ridleyana 50 O. paedunculata 41 O. trichocarpon 46 O. japonica O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 18 O. harrisiana 17 O. fucosa 64 O. harrisiana 18 O. harrisiana 27 O. fucosa 64 O. harrisana 18 O. harrisiana 27 O. fucosa 64 O. ridleyana 56 O. hiorrhiza sp. 35	ATTATTGACC CA. AATAAAGTAT	GATTTGGGTA	TATATGCAGA	AACCTTTCTC AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAGA 	1,200 GTTTGTATCG 1,300 TTTTTGAAAA GCTTCTTCG	12800 13600 14400 14400
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, rideyana 61 O, rideyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 17 O, rideyana 61 O, rideyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, rideyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, rideyana 62 O, padyatana Joosia umbellifera O, plumbea 6 O, harrisiana 17 O, fucosa 64 O, harrisiana 17 O, rideyana 51 O, pumbea 6 O, harrisiana 17 O, rideyana 61 O, rideyana 56 Ophiorrhiza sp. 35	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1,220 GTTTGTATCG	12800 13600 136000 136000 136000 10000000000
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, purnila O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, haquatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 61 O, tirchocarpon 46 O, purnila O, purnila O, seudofasciculata 62 O, pedunculata 41 O, tridleyana 56 Ophiorrhiza sp. 35 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae	ATTATTGACC CA. AATAAAGTAT	GATTTGGGTA	TATATGCAGA	AACCTTTCTC AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1,200 GTTTGTATCG 1,300 TTTTTGAAAA TTTTTGAAAAA	12800 13600 14400 14400
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, rideyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 72 O, rideyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 18 O, payatana Joosia umbellifera D, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, japonica 10 O, japonica 20 O, pedunculata 41 O, trichocarpon 44 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae	ATTATTGACC CA. AATAAAGTAT GATTAGGCTC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAA TGTACGTGTT GGGGGGGGCCCCCCCCCC	GTTTGTATCG	1200 1280 1360 1440 1400
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, pumila O, tridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pedunculata 41 O, trichocarpon 46 O, japonica O, haquatana Joosia umbellifera O, plumbea 6 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucleyana 56 Ophiorrhiza sp. 35 O, pumila O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica O, pumila O, ridleyana 52 O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	1,280 GTTTGTATCG	1200 1280 1360 1440
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 61 O, ridleyana 56 Ophiorrhiza sp. 35 O, purnila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, peedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 27 O, fucosa 64 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, iridleyana 50 O, purnila O, kuroiwae O, pedunculata 41 O, trichocarpon 46 O, japonica O, hayatana Joosia umbellifera O, plumbea 6 O, harrisiana 17 O, ridleyana 50 O, harrisiana 18 O, harrisiana 17 O, fucosa 64 O, harrisiana 18 O, harrisiana 18 O, harrisiana 17 O, ridleyana 61 O, ridleyana 61 O, ridleyana 61 O, ridleyana 52 O, pseudofasciculata 37 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harleyana 61 O, ridleyana 52 O, pseudofasciculata 62	ATTATTGACC CA. AATAAAGTAT GATTAGGCTC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAA G TGTACGTGTT G G G G G ACTTCCCAAG	1,200 GTTTGTATCG 1,300 TTTTTGAAAA TTTTTGAAAAA	12000 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 12800 13600 14400 14
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, rideyana 56 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 37 O, rideyana 52 O, pedunculata 41 O, trichocarpon 46 O, harrisiana 18 O, rideyana 61 O, rideyana 66 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 37 O, rideyana 52 O, psedunculata 41 O, trichocarpon 44 O, rideyana 52 O, pseudofasciculata 37 O, fucosa 64 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, indeyana 50 O, pumila 20 O, poumila 10 O, rideyana 50 O, pumila 27 O, fucosa 61 O, indeyana 50 O, pumila 0, kuroiwae O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 37 O, rideyana 52 O, pseudofasciculata 37 O, pseudofasciculata 43 O, pseudofasciculata 44 O, pseudofasciculata 45 O, pseudofasciculata 45 O, pseudofascicu	ATTATTGACC	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	T. A. 1300 T. A. 1300 T. A. 1300 CGGTTCTTCC	AAAAAAAAAAAA G TGTACGTGTT G G G G G ACTTCCCAAG	1,220 GTTTGTATCG	12000 12800 13600 14400 14
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, purnila O, ridleyana 52 O, pseudofasciculata 37 O, ridleyana 52 O, pedunculata 41 O, trichocarpon 46 O, japonica O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 66 Ophiorrhiza sp. 35 O, purnila O, seudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica D, seudofasciculata 37 O, ridleyana 56 O, harrisiana 18 O, harrisiana 17 O, ridleyana 52 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, harrisiana 18 O, harrisiana 17 O, fucosa 64 O, pumila O, sidelyana 52 O, pseudofasciculata 10 O, ridleyana 52 O, pedunculata 41 O, trichocarpon 46	ATTATTGACC CA. AATAAAGTAT	GATTTGGGTA	TATATGCAGA A. TTTCTTGTGT	AACCTTTCTC AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAGA 	1,200 GTTTGTATCG	12000 12800 13600 14400 14
O, plumbea 6 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, ridleyana 56 Ophiorrhiza sp. 35 O, purnila O, kuroiwae O, pseudofasciculata 37 O, ridleyana 52 O, pseudofasciculata 62 O, padunculata 41 O, trichocarpon 46 O, japonica O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, harrisiana 27 O, fucosa 64 O, indleyana 50 O, pumila O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pedunculata 62 O, pagonica O, harrisiana 18 O, harrisiana 18 O, harrisiana 17 O, ridleyana 50 O, pagonica O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 18 O, harrisiana 17 O, fucosa 64 O, ridleyana 50 Ophiorrhiza sp. 35 O, pumila O, kuroiwae O, pseudofasciculata 62 O, pseudofasciculata 37 O, ridleyana 50 O, pumila O, seculofasciculata 62 O, pseudofasciculata 62 O, pseudofasciculata 62 O, pedunculata 41 O, trichocarpon 46 O, japonica	ATTATTGACC CA. AATAAAGTAT	GATTTGGGTA	TATATGCAGA	AACCTTTCTC	ATTATCATAG	CGGTTCTTCC	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	GTTTGTATCG	12000 12800 13600 14400

		1,460		1,480	1,500	
O. plumbea 6	ACTTTTCGGG	GGGTATGTAG A	AAATCGAATT	TGGTATTTGG AAATTACTT	A TATCAACGAT CTGATC	AATC ATCAATGA 1518
O. harrisiana 18		A				
O. harrisiana 27		A				
O. fucosa 64		A				
O. ridleyana 61		A				
O. ridleyana 56		A				
Ophiorrhiza sp. 35		A				
O. pumila						
O. kuroiwae		A				
O. pseudofasciculata 37		A	<mark>G</mark>			
O. ridleyana 52		A	<mark>G</mark>			
O. pseudofasciculata 62		A	<mark>G</mark>			
O. pedunculata 41		A	<mark>G</mark>			
O. trichocarpon 46		A	<mark>G</mark>			
O. japonica		A	<mark>A.G</mark>			
O. hayatana		A	<mark>G</mark>			
Joosia umbellifera		<mark>A</mark> A	GGG	TTT		T 1518



APPENDIX D

The alignment of *Top*I nucleotide sequences of *Ophiorrhiza* species. Hyphens indicate gaps and dots represent identical nucleotides. Red characters represent different nucleotides.

		20		40		60		80	
O. fucosa 64	ATGGCTGTTG	AGGCCTGTAC	тастастаат	TTGATGGAGG	ATATGGAGGA	CGATGAGGGT	CCCGTGATTT	TCAAGAGAAG	80
0. harrisiana 27 Ophiorrhiza sp. 35									80 80
O. ridleyana 61									80
O. liukiuensis			C						80 80
O. pumila O. plumbea 6			c						80 80
O. pedunculata 41							<u>T</u>		80
O. Inchocarpon 46 O. japonica			C						80 77
O. pseudofasciculata 62							<u>T</u>		77
Camptotheca acuminata		T GT	C AAGAA A	ΑΤ.	.GTT	A.C.T.AG	AT.AG.	AC G	80
Catharanthus roseus		TC .	AAC.A	CGAT.	T	A T . AG	A.AG	. T A G . A	80
O fuence 64	TAACCCCCCCA	TCAAAGCAAA	ACCAAGCTAA	CTCCGAAAAA	AAGAAATTAT	CATTOCAGAA	GCATGTIGGA	CAATCIGCIG	160
O. harrisiana 27									160
Ophiorrhiza sp. 35 O. ridlevana 61	A			. <mark>G</mark>					160 160
O. ridleyana 56					<mark>G</mark> .				160
O. nukiuensis O. pumila									160
O. plumbea 6 O. pedunculata 41									160 160
O. trichocarpon 46				.		G			160
O. japonica O. pseudofasciculata 62	CA						G.A		157
O. pseudofasciculata 37 Camptotheca acuminata	A	c	TTTG			CT A G	G.A	A 664	157
Catharanthus roseus	TT.A		GT A	A C .	.GAGG.G.	ст	A.G. AG	A.T.A	160
		180 I		200		220 I		240 I	
O. fucosa 64 O harrisiana 27	TGCATACTTC	TGATGTACGT	CCGGCAAATG	GTGAAAGTTC	CAGTGCTCAA	AAGGGTAGAA	TACAACCC		228 228
Ophiorrhiza sp. 35									228
O. ridleyana 56									228
O. liukiuensis O. numila				•••••					228
O. plumbea 6									228
O. pedunculata 41 O. trichocarpon 46				A					228 228
O. japonica O. pseudofasciculata 62									225
O. pseudofasciculata 37									225
Camptotheca acuminata Catharanthus roseus	GA. GGTC.	G.C.	T.TA.C	стсс	CTC.	AG.	GGTC CC	ATCTAAGCCA	217
	00	260		280		300		320	240
O. fucosa 64		· · · · · · · · · · · ·		TCAGCCGAGA	CACCACCAGT	аааатсасст	CTCTCTAGCC	CAAAAGCATC	278
O, harrisiana 27 Ophiorrhiza sp. 35									278 278
O. ridleyana 61		••••							278
O. liukiuensis		<mark></mark>							278
O. pumila O. plumbea 6									278 278
O. pedunculata 41				A					278
O. incriocarpon 40 O. japonica				A					278
O. pseudofasciculata 62 O. pseudofasciculata 37				A					275
Camptotheca acuminata				TAAT	T.TTA.	GG	A.AG.A		266
Calnaraninus roseus	TCACAGGTTA	AATCTCCTCT 340	GTCTAGTCCA	CTTAC 360	TAG	T	AT.	.GTG 400	320
O. fucosa 64	AAATTCTTCA	GCCAAGGCAT	CGCCAATTAC	ATCTCCTGGA	GCAAATTCAA	AACTGTCTGC	TTCAGCAACC	AGTACTGCAA	358
O. harrisiana 27 Ophiorrhiza sp. 35									358
O. ridleyana 61			· · · · <u>·</u> · · · · ·						358
O. ridleyana 56 O. liukiuensis									358 358
O. pumila									358
O. pedunculata 41						<mark>C</mark>			358
O. trichocarpon 46 O. iaponica		111111111		G					358 355
O. pseudofasciculata 62						c			355
Camptotheca acuminata	C.A.TT		A G	G	с.т.	GG T CAG	C. T. GAT	GA.CGGT	334
Catharanthus roseus	CA	AA 420	.TGT.	TTTACC.		CACA.	C.GG.GT	TTAG.TG. 480	400
O, fucosa 64	AACAGCAGAA	CCATCATAAA	тсттссосто	CTTTTATTGA	GCCAAAGCCT	TCAGTTCCT-	<mark>с</mark> стос	TAAAGCAAAC	432
O. harrisiana 27 Ophiorrhiza sp. 35									432
Ophiomiza sp. 35 O. ridleyana 61			T				G		432
O. ridleyana 56 O. liukiuensis							G		432
O. pumila					C		G		432
O. pluribea 6 O. pedunculata 41							G		432
O. trichocarpon 46							•••••G		432
O. pseudofasciculata 62							G		429
Camptotheca acuminata	TTCATC	TA.A.G.T	A.AAT.AA	T.GAG	.GAGGAA	. TG AA		.G.GCT.T	429 411
Catharanthus roseus	CCTTT	A	G.A	T.AAG	.GAT.G		GATTGGT.	.GC.AT. TT	480
O fucosa 61	AGCAACTOCO	AGAGCTCTCA	TGATGAGAAA	CCATTGATCO	CTAGGACTTC	TGGTTGCTCA	TCCAAGCCAA		510
O. harrisiana 27	AGCAACTCCG								512
Ophiorrhiza sp. 35 O. ridlevana 61							T		512 512
O. ridleyana 56					C				512
			• • • • • • • • • • •						100 CT - 2
O. pumila			· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	.č				512
O. pumila O. plumbea 6 O. pedunculata 41			· · · · · · · · · · · · · · · · · · ·		.cŢ				512 512 512 512
O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·			. C		· · · · · · · · · · · · · · · · · · ·		512 512 512 512 512 512
O. plumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62					. C				512 512 512 512 512 512 509 509
O. pumila O. piumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptofhaca acuminata					.CT T T T				512 512 512 512 512 512 509 509 509

								116	
		580		600		620 I		640 I	
O. fucosa 64	TGCTAATAAG	GAGGGTGGTG	ATTCTTGTTC	AATTCGAAGG	CCAGTTGTCG	AAGAAAG		TGAAGATT 57	77
O. harrisiana 27 Onbiorrhiza sp. 35					•••••				17 77
O. ridleyana 61					A . T .				77
O. ridleyana 56 O. liukiuensis					A . T .				17 77
O. pumila					A				77
O. plumbea 6 O. pedunculata 41					A GA				17 77
O. trichocarpon 46					A			57	77
O. japonica O. pseudofasciculata 62					A				74 74
O. pseudofasciculata 37		CATTCATCA		CCC TC TT	A	CC CAAAT	CANTAGATAT	57	74
Catharanthus roseus	A.TG.GC.A		.GC			A			98
		660 I		680 I		700 I		720 I	
O. fucosa 64 O barrisiana 27	CAGAGGATGA	AAAACCTCTG	TCACGGAGGT	TCCCATCAAA	GTCGAGTTTA	GGGGAATCTA	CTAGCAAGTC	TTACGACTCG 65	57 57
Ophiorrhiza sp. 35								65	57
O. ridleyana 61 O. ridleyana 56		<mark>G</mark>						A 65	57 57
O. liukiuensis								A 65	57
O. pumia O. plumbea 6								. C A 65	57 57
O. pedunculata 41				A				T A 65	57
O. japonica				A				TA 65	54
O. pseudofasciculata 62 O. pseudofasciculata 37			T.C	A				TA 65	54 54
Camptotheca acuminata	.GT	TT. GT.	TCA.A	G. TG.	.G.C.A.GC	AC	GTA A	G T A T 64	12
Catharanthus roseus	. C T	A	TCTA.	G.T	CGG.T	G	G.	TT.67	78
O, fucosa 64	GATGAAAAGA	AGCCATTAGC	AGCAAAAGTT	CAACAAAATG	GTTCTGCTTC	GCGTGACGGT	CCAATGAACA	AATCTGCTTC 73	37
O. harrisiana 27									37
Opniorrniza sp. 35 O. ridleyana 61									37
O. ridleyana 56			· A						37
O. pumila									37
O. plumbea 6 O. pedunculata 41				c					37 37
O. trichocarpon 46				c		A			37
O. japonica O. pseudofasciculata 62				G					34 34
O. pseudofasciculata 37 Camptotheca acuminata	· · · · · · · · · · · · · · · ·	AA T G T	с		A ATCAA	TA G GTA		G T AT 73	34 13
Catharanthus roseus	TG T	. A T	TAAC		AAT	AT	А.ССТ.	A.T.AAA 75	58
O fucosa 64	TTTGTCAAAT	AAAAGAGGTC	CACCCACCT	TAAATCCTTC	AATCAGTOTT	CACTTAACAA	ACCTAACCTT	TCTAGTCCAA	17
O. harrisiana 27								8	17
Opniormiza sp. 35 O. ridleyana 61									17
O. ridleyana 56	· · · · ·] · · · ·	. <mark></mark>					. <u>G</u>	8	17
O. nukidensis O. pumila		c							17
O. plumbea 6 O pedunculata 41		c							17
O. trichocarpon 46		c						8	17
O. japonica O. pseudofasciculata 62	G					G			14
O. pseudofasciculata 37 Camptotheca acuminata		c	c			<mark>.</mark>			14
Catharanthus roseus	.A		тта.	CA	A.TA.	. GG	G	AGT 8	38
		900 I		920 I		940 I		960 I	
O. fucosa 64 O. harrisiana 27	TCACATCCAT	CAGCAACAAG	CAGGCAGCTG	TAAAACCAGA	ACCA	AAGGCTGAGG	ATGATGATGA	TAATATTCCT 8	91 91
Ophiorrhiza sp. 35									91
O. ridleyana 56									91 91
O. liukiuensis O. numila									91 91
O. plumbea 6								8	91
O. pedunculata 41 O. trichocarpon 46									91 91
O. japonica					GAACCA	A		8	94
O. pseudofasciculata 37								8	88
Camptotneca acuminata Catharanthus roseus	CT C.AG. CTG C.G.C	AT		AG					37 03
		980		1,00	0	1,020)	1,040	
O. fucosa 64	TTATCTCAAC	GAGGGAAGAA	GTCACAAACT	GCAGCCAGTA	AACCAAAGGA	TGAGGATGAT	GATAATGCTC	CAATATCCCA 9	71
Ophiorrhiza sp. 35									71 71
O. ridleyana 61 O. ridleyana 56							<mark>C</mark>		71
O. liukiuensis									71
O. pumila O. plumbea 6									71
O. pedunculata 41				A			<u>T</u>		71
O. inchocarpon 40				<mark>A</mark>			· · · · · · · · · · · · · · · · · · ·		74
O. pseudofasciculata 62 O. pseudofasciculata 37			AT	A		c			68 68
Camptotheca acuminata					GG G.T.	.AGT		.TTT8	78
Califarantinus roseus		1,060	,	1.08	0	1.10))	.C.I.I.9	23
O. fucosa 64	AAGAATCAAG	AAGTCACCAG	CTTCTGTTAG	CAAATCATCA	GCACCTGTAA	AAAAAGCATC	TACAGTTGTA	TCACCTTCTC 1	051
O. harrisiana 27 Onbiorrhiza sp. 35									051
O. ridleyana 61			Τ						051
O. ridleyana 56 O. liukiuensis									051 051
O. pumila				·····				10	05
O. piumpea 6 O. pedunculata 41				T					051 051
O. trichocarpon 46				Ţ				G	051
O. pseudofasciculata 62				<u>T</u>	. <u>T</u>			· · · · T · · · · · · 1	048
Camptotheca acuminata	GG	G. TA	.AA.GA	+	A.TG.AAA.C	T .A.	A.AC		048 58
(other outhing recours	-					~ ^ ^			

								117	
		1,140		1,160	,	1,180		1,200	
O. fucosa 64	TGAAAAAAGT	AAATAAGAAG	TCTAAGAAGG	TGATTAAAAA	AACAGCATAC	ACCAAGTCAT	CTAAAGTACC	тссбббттст	1131
O. harrisiana 27 Ophiorrhiza sp. 35		A							1131 1131
O. ridleyana 61		. A		G					1131
O. Idleyana 56 O. liukiuensis		A A							1131 1131
O. pumila		A							1131
O. pedunculata 41		A							1131
O. trichocarpon 46		A							1131
O. pseudofasciculata 62		A T						c	1128
Camptotheca acuminata		AT	TC A.	TG.G.	Т. АА	T.TG.	.AGG.	C	1128 1038
Ċatharanthus roseus		CA		. T G	CT. AAG. T	Τ	. A G	TA.C	1080
0.6		1,220		1,240	,	1,260		1,280 	
O. tucosa 64 O. harrisiana 27	GGCGAAGGTC	AAAAGTGGAC	TACTTTGGTC	CACAATGGTG	TGATGTTTCC	ACCTCCTTAC	AAGCCTCATG	GGGTTAAGAT	1211
Ophiorrhiza sp. 35 O ridlevana 61					т				1211
O. ridleyana 56					T T				1211
O. liukiuensis O. pumila									1211
O. plumbea 6					<u>T</u>				1211
O. trichocarpon 46					· · · · · · · · · · · · · · · · · · ·				1211
O. japonica O. pseudofasciculata 62		A			T		T		1214
O. pseudofasciculata 37					T				1208
Campioineca acuminata Catharanthus roseus		. G A A	CT	. т с	. A T	G		<mark>A</mark>	1118 1160
		1,300		1,320)	1,340)	1,360	
O. fucosa 64	GCTGTACAAG	CGGCAGCCCC	TTACTCTGAC	TCCCGAGCAA	GAGGAGGTTG	CGACAATGTT	TGCAGCGATG	CTAGATACTG	1291
Ophiorrhiza sp. 35		· A · · · · · · · · · ·							1291
O. ridleyana 61		A		· · · · · · · · · · · · · · · · · · ·			T		1291
O. liukiuensis		.AN		T			т		1291
O. pumila O. plumbea 6				•••••			тт.		1291
O. pedunculata 41		A					<u>T</u>		1291
O. Inchocarpon 40 O. japonica							· · · · · · · · · · · · · · · · · · ·		1291
O. pseudofasciculata 62 O. pseudofasciculata 37		· · · · · · · · · · A					T T		1288
Camptotheca acuminata	c	G. A AG		T A	A	. <mark>A</mark>	<u>T</u>	T	1198
Califarantinus roseus	A	G.ATG 1,380	GA.T	TA 1,400		. A	G.T	T.G 1,440	1240
O. fucosa 64	ATTACATGAA	TAAACCTCGT	TTTAAAGAGA	ACTTTTTTAG	TGACTGGAAA	AAGATACTGG	GAAAAAATCA	TACGATTCAG	1371
O. harrisiana 27 Ophiorrhiza sp. 35	C				•••••	G		G	1371
O. ridleyana 61		. <mark></mark> <mark></mark>				TA .			1371
O. ridieyana 56 O. liukiuensis	C	: <mark>:</mark> : : : : : : : : :				G		G	1371 1371
O. pumila O. plumbea 6		· · · · · · · · · · · ·							1371
O. pedunculata 41		C				G			1371
O. trichocarpon 46 O. iaponica						G			1371 1374
O. pseudofasciculata 62		.						.	1368
Camptotheca acuminata	С. ТС	C. T. AAG	c	T CA GGA	G.	т.	. <mark>G</mark> C	.GTA	1278
Catharanthus roseus	•••••	CA	CG	CG.		A .	. C C		1320
O. fucosa 64	AACTTGGAAG	1		1,46)	1,500	9	. GTC	
O. harrisiana 27 Ophiorrhiza sp. 35		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAGAGAAA	AAGAAACAAA	.GTC 1,520 I TGACTACAGA	1451
O ridlevana 61		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAGAGAAA A	AAGAAACAAA . <mark>G</mark>	.GTC 1,520 I TGACTACAGA	1451 1451 1451
o interograma or		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAGAGAAAA A	AAGAAACAAA	.GTC 1.520 I TGACTACAGA	1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAGAGAAA . A	AAGAAACAAA .G	GTC. 150 TGACTACAGA	1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAGAGAAA . A	AAGAAACAAA .G	.GTC. 1,520 TGACTACAGA	1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pequnculata 41		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAGAGAAA . A	AAGAAACAAA .G	.GTC. 1.520 TGACTACAGA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAGAGAAA A	AAGAAACAAA .G	ISTO INTERNATIONAL INTERNATIONALIZIANA INTERNATIONAL INTERNATIONALIZIA INTERNATIONALI INTERNATIA INTERNATIONALIZIA INTERNATIA INTERNATIA INTERNATIA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 62		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAGAGAAA AAAAAA	AAGAAACAAA .G	ISTO ISTO	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. pseudofasciculata 62 O. pseudofasciculata 37 Camptotheca acuminata		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAAGAAAA A	AAGAAACAAA .G	GTC.	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. pelumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAAGAAAA A	AAGAAACAAA G	GTC.	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. pseudofasciculata 62 O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64		ACTGTGACTT	TGGCCCTATA		ATCAGCAAGA	AAGAAGAGAAA A	AAGAAACAAA .G	.GTC. 1.520 1.	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. harrisiana 27		ACTGTGACTT	TGGCCCTATA CAC. A. CA. T ATGAGAGAGACT	TATGAGTGGC	ATCAGCAAGA ATCAGCAAGA AGC AGC GAGAAATATA	AAGAAGAGAAA A	AAGAAACAAA GG. GG. AG. TGTTGATGGT	.GTC. 1,520 TGACTACAGA 	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. pseudofasciculata 62 O. pseudofasciculata 37 Camptotheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorthiza sp. 35 O. ridleyana 61		ACTGTGACTT	TGGCCCTATA CAC. A. C CAC. A. T ATGAGAGAGACT A.	TATGAGTGGC	ATCAGCAAGA ATCAGCAAGA AGC AGC GAGAAATATA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G	GTCAAAGAGA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptotheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorthiza sp. 35 O. ridleyana 61 O. ridleyana 56 O. liukiuensis		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA ATCAGCAAGA AGC AGC GAGAAATATA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.	GTCAAAGAGA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. ridleyana 56 O. jukiuensis O. pumila		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA ATCAGCAAGA AGC AGC GAGAAATATA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.	GTCAAAGAGA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. ridleyana 56 O. liukiuensis O. plumbea 6 O. pedunculata 41		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC	ATCAGCAAGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.	GTCAAAGAGA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. piumbea 6 O. pedunculata 41 O. richocarpon 46 O. podunculata 41		ACTGTGACTT	TGGCCCTATA CAC. A. CA. T ATGAGAGAGACT A. A. A	TATGAGTGGC	ATCAGCAAGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.	GTCAAAGAGA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 61 O. ridleyana 56 O. liukiuensis O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica 20 O. pseudofasciculata 62		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC 	ATCAGCAAGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.	GTCAAAGAGA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 61 O. ridleyana 56 O. liukiuensis O. piumbea 6 O. pedunculata 41 O. richocarpon 46 O. japonica O. pseudofasciculata 37 Camptofheca acuminata		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC 	ATCAGCAAGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.G.	GTCAAAGAGA	1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 61 O. ridleyana 56 O. ridleyana 56 O. plumbea 6 O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC TATGAGTGGC 	ATCAGCAAGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 61 O. ridleyana 56 O. liukiuensis O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptoftaca acuminata Catharanthus roseus		ACTGTGACTT	TGGCCCTATA	TATGAGTGGC TATGAGTGGC 	ATCAGCAAGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 61 O. ridleyana 56 O. liukiuensis O. plumbea 6 O. plumbea 6 O. pedunculata 41 O. pseudofasciculata 62 O. pseudofasciculata 37 Camptoftacea acuminata Catharanthus roseus O. pseudofasciculata 37 Camptoftacea acuminata Catharanthus roseus	AGAAAAGAAG AGAAAAGAAG AGGTCGGTAA	ACTGTGACTT	TGGCCCTATA 	TATGAGTGGC TATGAGTGGC 	ATCAGCAAGA ATCAGCAAGA AGC AGC GAGAAATATA GAGAAATATA GAGAAATATA AGC GAGAAATATA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 66 O. liukiuensis O. pidleyana 56 O. ridleyana 56 O. ridleyana 56 O. ridleyana 56 O. ridleyana 56 O. pidunculata 41 O. prichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptoftacca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 54 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 54	AGAAAAGAAG AGAAAAGAAG AGGTCGGTAA	ACTGTGACTT	TGGCCCTATA	TATGAGTGGC TATGAGTGGC 	ATCAGCAAGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 66 O. liukiuensis O. piumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56	AGGTCGGTAA	ACTGTGACTT	TGGCCCTATA	TATGAGTGGC TATGAGTGGC A A C C A C C A C C A C A A A A A A	ATCAGCAAGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 61 O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. pumila	AGGTCGGTAA	ACTGTGACTT	TGGCCCTATA CAC A	TATGAGTGGC TATGAGTGGC A CAGCTAGAG CCAGCTAGAG A A A A A A A A A A A A A A A A A	ATCAGCAAGA ATCAGCAAGA AGC AGC AGC GAGAAATATA AGC AGGGCGTGGA AGGGCGTGGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 61 O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. pseudofasciculata 37 Camptoftheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. pumila O. ridleyana 56 O. liukiuensis O. pumila	AGGTCGGTAA	ACTGTGACTT	TGGCCCTATA	TATGAGTGGC TATGAGTGGC A A CCAGCTAGAG CCAGCTAGAG A A A A A A A A A A A A A A A A A	ATCAGCAAGA ATCAGCAAGA AGC AGC AGC GAGAAATATA AGC AGGGCGTGGA AGGGCGTGGA	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. piumbea 6 O. pedunculata 41 O. trichocarpon 46 O. pseudofasciculata 37 Camptofheca acuminata C. pseudofasciculata 37 Camptofheca acuminata C. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. priumbea 6 O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. ridleyana 61 O. plumbea 6 O. pedunculata 41 O. ptocarpon 46	AGGTCGGTAA	ACTGTGACTT	TGGCCCTATA	TATGAGTGGC TATGAGTGGC A A CCAGCTAGAG CCAGCTAGAG A A A A A A A A A A A A A A A A A	ATCAGCAAGA 	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. pidupana 56 O. liukiuensis O. pidupana 56 O. pidunculata 41 O. ridleyana 56 O. pidunculata 41 O. prichocarpon 46 O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. pidupana 56 O. liukiuensis O. pidupana 56 O. liukiuensis O. pidupana 56 O. liukiuensis O. pidunculata 41 O. ridleyana 64 O. pedunculata 41 O. ridleyana 64 O. pidunculata 41 O. ridleyana 66 O. pidunculata 41 O. pidunculata 41 O. pidunculata 41 O. paonica	AGGTCGGTAA	ACTGTGACTT	TGGCCCTATA	TATGAGTGGC TATGAGTGGC A A CCASCTAGAG CCASCTAGAG CCASCTAGAG A A A A A A A A A A A A A A A A A	ATCAGCAAGA 	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451
O. ridleyana 56 O. liukiuensis O. pumila O. plumbea 6 O. pedunculata 41 O. trichocarpon 46 O. japonica O. pseudofasciculata 62 O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. piumbea 6 O. pedunculata 41 O. trichocarpon 46 O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. pseudofasciculata 37 Camptofheca acuminata Catharanthus roseus O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. fucosa 64 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 56 O. liukiuensis O. piumbea 6 O. ridleyana 56 O. liukiuensis O. piumbea 6 O. piumbea 6 O. pedunculata 41 O. richocarpon 46 O. pedunculata 41 O. richocarpon 46 O. pedunculata 41 O. pseudofasciculata 62 O. pseudofasciculata 37 Capptofheca acuminata	AGGTCGGTAA	ACTGTGACTT	TGGCCCTATA	TATGAGTGGC TATGAGTGGC A A CCASCTAGAG CCASCTAGAG CCASCTAGAG A A A A A A A A A A A A A A A A A	ATCAGCAAGA 	AAGAAGAGAAA A.A.A.A.A.A.A.A.A.A.A.A.A.A.	AAGAAACAAA G		1451 1451 1451 1451 1451 1451 1451 1451

								118	
		1,70 I)	1.720 I	0	1,740 I	5	1,760	
O. fucosa 64 O. barrisiana 27	CGCATCCATC	CAAGGGATAT	TACCATAAAT	ATTGGAAAGG	ATGCTCCAAT	TCCTGAATGT	CCCATCCCCG	GTGAAAGATG	1691
Ophiorrhiza sp. 35									1691
O. ridleyana 56	G								1691
O. liukiuensis O. pumila	G G						т .		1691 1691
O. plumbea 6 O. pedunculata 41	G G								1691 1691
O. trichocarpon 46	G								1691
O. pseudofasciculata 62									1688
Camptotheca acuminata	T.TG.	.GTC	<mark>G</mark>	. .	с.т.	<mark>A</mark>	A T .		1688 1598
Catharanthus roseus	T G	. TT.C 1.78	· · · A · · · · · · · ·	1.800	· · · · · · · T · · ·	C	TG.TT .	1,840	1640
O. fucosa 64	GAA <mark>g</mark> gaagta	AGGAATGACA	ACACTGTGAC	ATGGTTAGCA	TATTGGAATG	АТССАСТААА	TCTAAAGGAA	TGCAAATATG	1771
O. harrisiana 27 Ophiorrhiza sp. 35	· · · · · · · · · · · ·								1771 1771
O. ridleyana 61 O. ridleyana 56	A		· · · · · · · · · · · · · · · · · · ·				. A		1771
O. liukiuensis	A				. T	A	A		1771
O. plumbea 6	A					A	A	сс.	1771
O. trichocarpon 46	A		Â			A	A	. Т	1771
O. japonica O. pseudofasciculata 62	A				.TT	A A	A	. T	1774 1768
O. pseudofasciculata 37 Camptotheca acuminata	A	G	A			A	A	.TG.C.	1768 1678
Catharanthus roseus	A	c.cc	. T G	G	.T	A.T	c	. T	1720
O, fucosa 64	TTTTTCTAGC	ACCCAGCAGT	ACCTTAAAGG	GGCAAAGTGA	CAAGGAGAAA	TATGAGAAAG	CAAGGCTCTT	AAAGGATTAC	1851
O. harrisiana 27 Onhiorrhiza sp. 35									1851
O. ridleyana 61		. G		T			G		1851
O. liukiuensis		.G							1851
O. pumila O. plumbea 6		.G					.		1851 1851
O. pedunculata 41 O. trichocarpon 46		.G							1851 1851
O. japonica O. pseudofasciculata 62									1854
O. pseudofasciculata 37 Camptotheca acuminata		.G							1848
Catharanthus roseus	GG	TG		.A.T	<mark>A</mark>			GC.II	1800
O fuence 64		1,94	TTATACTAAC	1,960		1,980		2,000	1021
O. harrisiana 27	ATACATGGCA	TCAGAGCTGC	ПАТАСТААС	GATTTTACTA	ATAATAAAGA		AAGCAAATAG		1931
Ophiorrhiza sp. 35 O. ridleyana 61				.G					1931 1931
O. ridleyana 56 O. liukiuensis		<mark>.</mark>							1931 1931
O. pumila O. plumbea 6		· · · · · · · · · · ·					G		1931 1931
O. pedunculata 41									1931
O. inchocal point 40 O. japonica									1934
O. pseudofasciculata 37									1928
Camptotneca acuminata Catharanthus roseus	AA.		A A	G.A. G	GCG	.ATC	CG		1835 1877
	1	2,02)	2,040 		2,060 I		2,080 I	
O. fucosa 64 O. harrisiana 27	TTATCTTATT	GACAAACTAG	CTCTGAGGGC	AGGCAATGAG	AAGGATGATG	ATGAAGCTGA	TACAGTTGGT	TGCTGCACAC	2011 2011
Ophiorrhiza sp. 35 O. ridleyana 61			· · · · · · · · · · · · · ·						2011 2011
O. ridleyana 56 O. liukiuensis			c						2011 2011
O. pumila O. plumbea 6									2011
O. pedunculata 41			c						2011
O. japonica			c						2014
O. pseudofasciculata 37			c						2008
Campioineca acuminaia Catharanthus roseus	cc			CAA			G		1915 1957
0.6		2,10		2,120		2,140 		2,160	
O. harrisiana 27	IGAAAGIAGA	AAATGTAGAA	cererecere		AAAGATTGAC	TTTATCGGTA	AGGATICCAT	IAGATATCAA	2091 2091
Ophiorrhiza sp. 35 O. ridleyana 61	G			C .		· · · · · · · · · · · · · · · · · · ·			2091 2091
O. ridleyana 56 O. liukiuensis				T	T	c			2091 2091
O. pumila O. plumbea 6					· · · · · · · · · · · ·	· · · · · · · · · · · ·			2091
O. pedunculata 41				<u>T</u>	<u>T</u>	c			2091
O. inclidealpoir 40 O. japonica					<u>T</u>	C			2091 2094
O. pseudofasciculata 37					T T	c			2088 2088
Camptotheca acuminata Catharanthus roseus			AAA 	GCT T	GT TT	C.T	. A A . A T	c	1995 2037
		2,18)	2,200)	2,220)	2,240	
O. fucosa 64 O. harrisiana 27	AATGAGGTCC	AGGTTGAACC	TGCTGTTTTC	AAGGCAATTC	AACAGTTCCG	AAGTGGGAAA	GAGGGTAGTG	AAGACCTTTT	2171 2171
Ophiorrhiza sp. 35 O. ridlevana 61			C						2171
O. ridleyana 56								C	2171
O. pumila									2171
O. pedunculata 41					. c			•••••	2171 2171
O. trichocarpon 46 O. japonica								. <mark>G</mark>	2171 2174
O. pseudofasciculata 62 O. pseudofasciculata 37							. T		2168 2168
Camptotheca acuminata Catharanthus roseus	TG	. A	.CG.A.		.GT.	CA	AGAG CCA	. T T	2075 2117
									- 3

								119	
		2,260 I)	2,280 I	D	2,300 I	1	2,320 I)
O. fucosa 64	TGACCGGCTT	GACACCAGTA	AACTAAATGC	TCATCTGAAG	GAACTCATGC	CTGGTCTTAC	CGCAAAAGTT	TTCCGTACAT	2251
Ophiorrhiza sp. 35									2251
O. ridleyana 61 O. ridlevana 56									2251 2251
O. liukiuensis									2251
O. plumbea 6									2251
O. pedunculata 41 O. trichocarpon 46									2251 2251
O. japonica O. pseudofasciculata 62									2254
O. pseudofasciculata 37									2248
Camptotneca acuminata Catharanthus roseus	G	T T	G		. GG T A	CC	A		2155 2197
		2,340)	2,360 	D	2,380	1	2,400	J
O. fucosa 64 O harrisiana 27	ATAATGCATC	AATAACATTA	GATGATATGT	TGAGTAAGGA	AACCAAGGGT	GGAAAGGTTG	CAGAGAAAGT	TGGGGTATAT	2331 2331
Ophiorrhiza sp. 35									2331
O. ridleyana 56				<mark>A</mark> .	. .	G		T	2331
O. liukiuensis O. pumila									2331 2331
O. plumbea 6					· · · · · · · · · · · · · · · · · · ·				2331
O. trichocarpon 46				Â.	T	G		T	2331
O. japonica O. pseudofasciculata 62						G	AC	T	2334 2328
O. pseudofasciculata 37 Camptotheca acuminata		T C T G			••••	G	AC		2328
Catharanthus roseus		TT			A	G.T	. T		2277
0.6		2,420		2,44		2,460		2,480	
O. harrisiana 27	CAACATGCAA	ATAAGGAGGT	IGCAATAATT		AGCGTACTGT		CACAGIGCAC	AAAIGICACG	2411
Ophiorrhiza sp. 35 O. ridlevana 61									2411 2411
O. ridleyana 56									2411
O. liukiuensis O. pumila	<mark>G</mark>				. A				2411
O. plumbea 6 O. pedunculata 41									2411 2411
O. trichocarpon 46									2411
O. pseudofasciculata 62							G.		2414
Camptotheca acuminata					. A			A.GA.	2408 2315
Catharanthus roseus	G	. C	· · · · · · · · · · · · · · · · · · ·		<mark>G</mark>	ACC			2357
O fucosa 64	GTTGAATGAA	AAGATAGACG	AACTTAAGAC	TGCTCTGGAA	GAATTGAAAA	CCGATTIGIC	TAGGGCCAAA	AAGGGTAAGC	2491
O. harrisiana 27									2491
Opniormiza sp. 35 O. ridleyana 61									2491 2491
O. ridleyana 56 O. liukiuensis		: <mark>.</mark> 		. A					2491 2491
O. pumila									2491
O. pedunculata 41									2491 2491
O. trichocarpon 46 O. japonica				A			T		2491 2494
O. pseudofasciculata 62 O. pseudofasciculata 37									2488
Camptotheca acuminata			.G. AGG	CAT T	.GGC	. <mark>G</mark> G .	AG	A G	2395
Califarantinus roseus	ACC	2,580))	TGG 2,60	0GT	. T C 2,620	. C T	AG 2,640	2437
O. fucosa 64	CACCAT	CAAAGGGT	GATGATGGG	AGCCAAAGAG	GAATTTGAAC	CCTGAAGC -			2543
O. harrisiana 27 Ophiorrhiza sp. 35									2543 2543
O. ridleyana 61 O. ridleyana 56		A.	.c			T	GAGTGCTCTG	GIICCIIGII	2543
O. liukiuensis	G		.c			T			2543
O. plumbea 6	. <mark>G</mark>		.c						2543 2543
O. pedunculata 41 O. trichocarpon 46	T	1	. C A						2543 2543
O. japonica O. pseudofasciculata 62		••••••	. C A				CAGIGCICIO	GIICCIIGII	2546
O. pseudofasciculata 37			.cA						2540
Catharanthus roseus	G. TAAA	GT AA	. C A	. A A	A	T			2447
		2,660 I	· /	2,68	D	2,700	3	2,720 I)
O. fucosa 64 O. harrisiana 27						GCTTGA	GAGAAAGATA	GCACAAACCA	2569 2569
Ophiorrhiza sp. 35									2569
O. ridleyana 56	GTAAGATGTA	CATCGTGTAT	GCTTGGTATA	ACTGATGATC	AATGTTCTTT	TCAGA			2645
O. liukiuensis O. pumila						A			2569 2569
O. plumbea 6 O. pedunculata 41						A			2569 2569
O. trichocarpon 46						A			2569
O. pseudofasciculata 62	GTAAGATGTA	CATCGTATAT	GTTTGGTATA	ACTGATGATC	AATGTTGTTT	TCAGA	G	A	2642
Camptotheca acuminata						A	G	AA.	2566 2473
Catharanthus roseus						•••••AG	<mark>A</mark>	T	2521
() fucosa 64	АТССТААААТ	TGAGAAGATG	GAACGTGACA	AAAAGACCAA	AGAGGATTTG	AAAGCCGTAG	CTTTGAGCAC	GTCAAAGATC	2649
O. harrisiana 27									2649
Opniorrhiza sp. 35 O. ridleyana 61									2649 2649
O. ridleyana 56 O. liukiuensis				G		A	G		2725
O. pumila						••••			2649
O. piumbea 6 O. pedunculata 41				G		A	G		∠649 2649
O. trichocarpon 46 O. japonica				G		A	G		2649 2652
O. pseudofasciculata 62 O. pseudofasciculata 37				G		A	G		2722
Camptotheca acuminata	AG			.GG A	GGCC	AAT.	.AG.T	A	2553
Canaranana 100000		A			JA			A	2001

								120	
		2,820)	2,840)	2,860	1	2,880	
O. fucosa 64	AGTTACCTTG	ATCCTAGAAT	AACTGTTGCA	TGGTGCAAGC	GTCAAGAGGT	TCCAATTGAG	AAGATGTTCA	ACAAGTCTCT 272	29
O. harrisiana 27					<mark>.</mark>		<mark>.</mark>	272	29
Ophiorrhiza sp. 35					<mark>.</mark>		· · · · · · · · · · · ·	272	29
O. ridleyana 61					<mark>.</mark>		· · · · · · · · · · · ·	272	29
O. ridleyana 56	. <mark>A</mark>		G		C		A	280	05
O. liukiuensis					C <mark>A</mark>		A	272	29
O. pumila					<mark>.</mark>		<mark>.</mark>	272	29
O. plumbea 6		G			C		· · · · · · · · · · · ·	272	29
O. pedunculata 41	. <mark>A</mark>		G		C		A	272	29
O. tricnocarpon 46	. A		G		C		A	272	29
O. japonica	. <mark>A</mark>				C		A	273	32
O. pseudolasciculata 62	. A		G		C		A		02
Comptethese seuminets	· A		G		C		A	272	26
Campioineca acuminaia	C	<mark>G</mark>	CGC		.ATA	C	G.A		33
Califarantinus Toseus	. A		AC		.CTA		A	A268	81
		2,900)	2,920)				
O. fucosa 64	TCTGGCGAAG	TTTGCCTGGG	CCATGGATGT	TGATCCCAGC	TTCAGATTTT	GA 2781			
O, harrisiana 27			A	CA T		2766			

O. fucosa 64 TCTGGCGAAG TTTGCCTGGG CCATGGATGT TGATCCCAGC TTCAGATTIT GA 2781 O. harrisiana 27 Ophiorrhiza sp. 35 O. ridleyana 61 O. ridleyana 66 O. plumbea 6 O. plumbea 6 O. pedunculata 41 O. pedunculata 41 O. pseudofasciculata 62 O. pseudofasciculata 37 Camptotheca acuminata Catharanthus roseus G. T. A. A. T. A. T. A. T. C. 2783



120

APPENDIX E

The alignment of Topl amino acid sequences of *Ophiorrhiza* species and other organisms. Hyphens indicate gaps. Red characters represent different residues.

Homo seguesting Mark Latter Latt			20		40		60		80	
Campachanity fields a state of the constrained and state of the constraine	Homo sapiens	MSGD		EPI No			DENDAKENKK	EKDREKS		47
C. Paching MALEATT -: LIEUMENDE FY IFRRAFT, SCROLARSE, KILLGOWY, GLAVITER, PACERLAG, KELLGOWY, GLAVITER, PACERLAG, K	Catharanthus roseus	MAVEACPTPN	LRDDMDEDDE	PIVFKRNNSA	SKQSQTNSET	RKVSSQKRDG	QSVRQIYDSR	PANGESSSGQ	KGRMVPPSKP	80
D. De Consideration of the second of the sec	O. japonica	MAVEACTP - N	LMEDMEDDEG	PVIFKRSNPA	SKQNQANSEK	KKLSLQKHVG	QSAVHTSDVR	PANGESSSAQ	KGRIQPSAK -	78
C. pediatellas Declaration	O. pseudotasciculata	MAVEACTT - N	LMEDMEDDGG	PVIFKRSKPA	PKQNQANSEK	KKLSLQKQDG	QSAVHTSDVR	PANGESSSAQ	KGRIQPSAK -	78
C. Juning M. ALTER LEUKELT I. LEUKELT V. FARANCE S. J. J. STRAND S. SOLGARSK K. LLGOVY G. ANTIENT F. SOLGARSK K. LLGOVY G. ANTIENT F	O pedunculata	MAVEACITIN		PVIEKRSNPA	SKONDANSEK	KKLSLQKHVG	QSAVHISDVR	PANGENSSAQ	KGRIQPSAK -	79 79
Opinion/torsyn Description	O. plumbea	MAVEACTTPN	LMEDMEDDEG	PVIFKRSNPA	SKQNQANSEK	KKLSLQKHVG	QSAVHTSDVR	PANGESSSAQ	KGRIQPSAE -	79
C. And The Art of the	Ophiorrhiza sp. 35	MAVEACTTTN	LMEDMEDDEG	PVIFKRSNPA	SKQNQANSEK	KKLSLQKHVG	QSAVHTSDVR	PANGESSSAQ	KGRIQPSAE -	79
C. minuses with a first second	O. harrisiana	MAVEACTTTN	LMEDMEDDEG	PVIFKRSNPA	SKQNQANSEK	KKLSLQKHVG	QSAVHTSDVR	PANGESSSAQ	KGRIQPSAE -	79
C. Johnson W.V.A.T.T.N. LEDUCODE V.V.FREMA, SCALARLEY, KLUDONE SKUTTOV, FARESSKA CRUTCAL, SCALARLEY, KLUDONE SKUTTAL, SCALARLEY, SCALA	O. Ilukiuerisis O. ridlevana	MAVEACTTPN	LMEDMEDDEG	PVIFKRSNPA	SKONQANSEK	KKLSLOKHVG	QSAVHTSDVR	PANGESSSAQ	KGRIQPSAE -	79
0. https://www.scrim.list.com/org/intermed/scrime/scrim/scrime/scrim/scrime/scrime/scrime/scrime/scrime/scrime/scrime/s	O. pumila	MAVEACTTPN	LMEDMEDDEG	PVIFKRSNPA	SKONGANSEK	KKLSLOKHVG	OSAVHTSDVR	PANGESSSAG	KGRIOPSAE-	79
Campanes	O. fucosa	MAVEACTTTN	LMEDMEDDEG	PVIFKRSNPA	SKQNQANSEK	KKLSLQKHVG	QSAVHTSDVR	PANGESSSAQ	KGRIQPSAE -	79
Honon sighted	Camptotheca acuminata	MAVQACVKEK	MMDEIDEDDE	PLVFKRSNAT	SKHNQLNSDT	KK-SSQRYDG	QSGRQVSDVP	STNGQSSSAS	KGKTTK-	75
Attract supports Company of the supports <thcompany of="" supports<="" th="" the=""> <thcompany of="" supports<="" td="" the=""><td></td><td></td><td>100</td><td></td><td>120</td><td></td><td>140 I</td><td></td><td>160 I</td><td></td></thcompany></thcompany>			100		120		140 I		160 I	
Campache according to the second seco	Homo sapiens			KHSNSE	HKDSE-KKHK	EKEKTKHKDG	SSEKHKDKHK	D		83
0. pepulotisSiculius 0. pepulotisSiculius	O ianonica	SQVKSPLSSP	PSKPSQVKSP		AKKSPVSSLP	ANSKPSISGS	STAKOONHHK	SAIVIKEEKS	SPLRLASA	15
C. frichcargon C. frichcargon	O. pseudofasciculata	TP	P VKSP	LSSPKASNSS	AKASPITSPG	ANSKPSASAT	STAKQQNHHK	SSAAFIEPKP	SV - PAAKANS	14
C. peduchalan O. horizolan O. horizolan O	O. trichocarpon	TP	P VKSP	LSSPKASNSS	AKASPITSAG	ANSKPSASAT	STAKQQNHHK	SSAAFIEPKP	SV - PAAKANS	14
Ophomy 20, 20, 30, 30 Image: Strange S	O. pedunculata	TP	PVKSP	LSSPKASNSS	AKASPITSPG	ANSKPSASAT	STAKQQNHHK	SSAAFIEPKP	SV - PAAKANS	14
O. hardbards	Onhiorrhiza sp. 35	TP	PVKSP	LSSPKASNSS	AKASPITSPG	ANSKLSASAT	STAKQQNHHK	SSAAFIEPKP	SV - PAAKANS	14:
O. Ruduessis T.P. V.V.SP Lask Franks AAAP TEPC ANSKL SAAL TAXOOHINI SAAL EPC V.P.AAAAS Campathue O. Ruccas T.P. V.V.SP Lask KAAP TEPC ANSKL SAAL TAXOOHINI SAAL SAAL FTP V.P.AAAAS Campathue Same FTP V.V.SP Lask KAAP TEPC ANSKL SAAL STAKOOHINI SAAL FTP V.P.P.AAAAS Campathue Same FTP V.V.SP Lask KAAP TEPC ANSKL SAAL STAKOOHINI SAAL SAAL FTP V.P.P.AAAS Campathue Campathue Same	O. harrisiana	TP	PVKSP	LSSPKASNSS	AKASPITSPG	ANSKLSASAT	STAKQQNHHK	SSAAFIEPKP	SV - PPAKANS	14
C. notes	O. liukiuensis	T P	PVKSP	LSSPKASNSS	AKASPITSPG	ANSKLSASAT	STAKQQNHHK	SSAAFIEPKP	SV - PAAKANS	14
Campadhes and an	O. ridleyana	TP	PVKSP	LSSPKASNSS	AKASPITSPG	ANSKLSASAT	STAKQQNHHK	SSAAFIEPKP	SV - PAAKANS	14
Campadheca acuminata	O fucosa	TP	P	LSSPKASNSS	AKASPITSPG	ANSKLSASAT	STAKQQNHHK	SSAAFIEPKP	SV-PAAKANS	14
Homo saplems NEXEKANCE Environmentation Participation Participat	Camptotheca acuminata	LS	S MKSP	IASPKASTSF	AKASPV	ANPKVSSSSD	DRSKHSSKQN	TINVVKEEKE	LVNPAAEPYG	13
Homo sapiens -ROKKINKE ENG Status	-		180		200		220		240	
Catharanhus rössus 0. pp. 0.	Homo sapiens	RDKEKRKE	EK VRASG	DAKIKKEK	ENGE	SSP POIK	DEPEDDGYFV	P		12
O. Jeponica	Catharanthus roseus	IINDSEDSDD	GKPLSLRHSA	SSSKGNTNQV	SK EGS -	КР	E-NEDSDDEK	PLSSRFPLKS	GV GESTSKAY	22
C. populations	O. japonica	NSESSDD	EKPLSARTFG	CSSKGKSNHA	NK EGGD	SCSIRRRVIE	E-SEDSEDEK	PLSRRFTSKS	SLGESTSKSY	21
C. predunctidati	0. pseudolasciculata	NSESSDD	EKPLSARTEG	CSSKGKSNHA	NK EGGD	SCSIRRPVIE	E-SEDSEDEK	PLSCRFTSKS	SLGESTSKSY	21
O. plumbes	O. pedunculata	NSESSDD	EKPLSARTEG	CSSKGKSNHA	NK EGGD	SCSIRRPVIE	E-SEDSEDEK	PLSRRFTSKS	SLGESTSKSY	21
Opionthiza 53	O. plumbea	NSESSDD	EKPLIARTEG	CSSKGKSNHA	NK EGGD	SCSIRRPVIE	E-SEDSEDEK	PLSRRFPSKS	SLGESTSKSY	21
C. mailantii	Ophiorrhiza sp. 35	NSESSDD	EKPLIARTSG	CSSKGKSNHA	NK EGGD	SCSIRRPVVE	E-SEDSEDEK	PLSRRFPSKS	SLGESTSKSY	21
O indivigual	O. harrisiana O. liukiuensis	NSESSDD	EKPLIARTSG	CSSKGKSNHA	NK EGGD	SCSIRRPVVE	E-SEDSEDEK	PLSRRFPSKS	SLGESTSKSY	21
O. pliming	O. ridlevana	NSESSDD	EKPLIARTSG	CSSMGKSNHA	NK EGGD	SCSIRRPVIE	E-SEDSEDEK	PLSRRFPSKS	SLGESTSKSY	21
C. JILCOSS	Q. púmila	NSESSDD	EKPLIARTSG	CSSKGKSNHA	NK EGGD	SCSIRRPVIE	E-SEDSEDEK	PLSRRFPSKS	SLGESTSKSH	21
Campandeca acuminata	O. fucosa	NSESSDD	EKPLIARTSG	CSSKGKSNHA	NK EGGD	SCSIRRPVVE	E-SEDSEDEK	PLSRRFPSKS	SLGESTSKSY	21
Homo sapiens PKED KPL . KTW KER KKRA KLONGSARE DGGYNKYSME SNKRPPGLY SSNGLSYKKP KLESYTATTS KKRASYKPEP - KAEDDDN O. pseudofisciculate bockketa k kuonisase Dgornkkast snkrppgek sludskyke klesytatis knoasvkPep - KAEDDDN O. pseudofisciculate bockketa k kuonisase Dgornkkast snkrppgek sludskyke klesytatis knoasvkPep - KAEDDDN O. pdornulate bockketa k kuonisase Dgornkkast snkrppgek sludskyke klesytatis knoasvkPep - KAEDDDN O. pdornulate bockketa k kuonisase Dgornkkast snkrppgek sludskyke klesytatis knoasvkPep - KAEDDDN O. pdornulate bockketa k kuonisase Dgornkkast snkrppgek sludskyke klesytatis knoasvkPep - KAEDDDN O. plukuensis DSGEKKPLA KUONISASE DGOrnkkast snkrpgek sludskyke klesytatis knoasvkPep - KAEDDDN O. lukuensis DSGEKKPLA KUONISASE DGOrnkkast snkrpgek sludskyke klesytatis knoasvkPep - KAEDDDN O. lukuensis DSGEKKPLA KUONISASE DGOrnkkast snkrpgek sludskyke klesytatis knoasvkPep - KAEDDDN O. lukuensis DSGEKKPLA KUONISASE DGOrnkkast slukrapgek sludskyke klesytatis knoasvkPep - KAEDDDN O. lukuensis DSGEKKPLA KUONISASE DGOrnkkast slukrapgek sludssluke klesytatis knoasvkPep - KAEDDDN O. lukuensis DSGEKKPLA KUONISASE DGORNKASAS SNKRAPGEK SLUDSSLKKP KLESYTIS knoasvkPep - KAEDDDN O. lukuensis DSGEKKPLA KUONISASE DGORNKASAS SNKRAPGEK SLUSSSLKK KLESYTIS knoasvkPep - KAEDDDN O. delongen plens Cambardne klesytatis klesis tiskis tiskis tiskis tiskis tiskis klesis t	Campioineca acuminala	DSED	EKPLSARLFT	GLTKGSSNNA	NKGLINSSPA	SLPVPKPEIN	RYSDDSDDEI	PLSSKFRLKA	NAGTSTVKSY	21
<pre>rhomo sapiens PKED KPL +</pre>			260		280		300		320	
C. jpoordig UBBENKELAA KUUNGGASAR DCHWIKAGL SHKRPFOLK SUNGSVKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. trichocarpon DSDEKKPLAA KUOPIGSASR DCFWIKSASL SHKRPFOLK SLNGSUKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. plunibg DSDEKKPLAA KUOPIGSASR DCFWIKSASL SHKRPFOLK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. plunibg DSDEKKPLAA KUOPIGSASR DCFWIKSASL SHKRPFOLK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUOPIGSASR DCFWIKSASL SHKRPFOLK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUONGSASF CFWIKSASL SHKRPFOLK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUONGSASF CFWIKSASL SHKRPFOLK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUONGSASF CFWIKSASL SHKRPPCKK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUONGSASF CFWIKSASL SHKRPPCKK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUONGSASF CFWIKSASL SHKRAPCEKK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUONGSASF CFWIKSASL SHKRAPCEKK SLNGSLKKP KLSSPITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUONGSASF CFWIKSASL SHKRAPCEKK SLNGSLKKV KLSSTITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDEKKPLAA KUONGSASF CFWIKSASL SHKRAPCEKK SLNGSLKKV KLSSTITSIS NKOAVKPEP - KAEDDDDN O. harrising DSDKKVK SATAFKPOE DDNNPISSI KKSKVKKSKVKK KLSSTITSIS NKOAVKVEP - KAEDDDDN O. JABONG SUBJEKKSKK GTAASKKKOE DDNNPISSI KKSFYEVKS SYVKKAST VYSSLKKVK KKT AYTKSSKVPF O. JBAONG SUBJEKKSKK GTAASKKKOE DDNNPISSI KKSFYEVKS SYVKKAST VYSSLKKVK KKT AYTKSSKVPF O. harrising IPLSORKKKS GTAASKKKOE DDNNPISSI KKSFYEVKS SYVKKAST VYSSLKKVN KKKKVKKT AYTKSSKVP O. harrising IPLSORKKS GTAASKKKOE DDNNPISSI KKSFYEVKS SYVKKAST VYSSLKKVN KKSKVIKKT AYTKSSKVP O. harrising SSCKKSK GTAASKKKOE DDNAPISSI KKSFYEVKS SYVKKAST VYSSLKKVN KKSKVIKKT AYTKSSKVP O. harrising SSCKKSKK GTAASKKKOE DDNAPISSI KKSFYEVKS SSAPVKKAST VYSSLKKVN KKSKVIKKT AYTKSSKVP O. harrising SSCKSKKKK GTAASKKKOE DDNAPISSI KKSFYEVKS SSAPVKKAST VYSSLKKVN KKSKVIKKT AYTKSSKYP O. harrising SSCKSKKKS GTAASKKKE DDNAPISSI KKSFYEVKKS SSAPVKKAS	Homo sapiens	PKEDIKPL			KRPRDE	DDADYKPK	KIKTEDT	KKEK	KRKLEEEED -	17
O. pseudolásticulato SDEKKPLAK KVODNEGASR DOPMNIKASAL SNKRPPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. peduncilato BDEKKPLAK KVONGGASR DOPMNIKASAL SNKRPPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. plumbed BDEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. plumbed BDEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. plumbed BDEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. plumbed BDEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. plumbed BDEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. fulciosa DSEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. fulciosa DSEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. fulciosa DSEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. fulciosa DSEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. fulciosa DSEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. fulciosa DSEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPECK SLNOSSUKKP KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. fulciosa DSEKKPLAK KVONGGASR DOPMNIKASAL SNKRAPEKK SLNOKSUK KLSSPITSIS NKOAAVKPEP - KAEDDDDN O. fulciosa DSEKKPLAK KVONGSAR DOPMNIKASAL SNKRAPEKK SLNOKSUK KLSSPITKISK VKVKK KKKKK KKKKK KKKK KKKKKKKKKK KKKKKK	O. iaponica	DSDEKKPLAK	KUOONGSVSR	DGPMNKSASI	SNKRPPGDIK	SINGLSVKKP	KLSSVIAPIN	NKOAAVKPEP	KAEDDDD-	29
0. Inchocarport DSDEKKPLAA KVQPNGSASE DEPNIKSAGL SNKRPPCEVK SLNGSSLKKY KLSPITSIS NKGAAVKEEPKAEDDDDN Ophol Sterkplaa KVQPNGSASE DEPNIKSAGL SNKRPPCEVK SLNGSSLKKY KLSPITSIS NKGAAVKEEP	O. pseudofasciculata	DSDEKKPLAA	KVQQNGSASR	DGPMNKSASL	SNKRPPGEAK	SLNQSSVKKP	KLSSPITSIS	NKQAAVKPEP	KAEDDDDN	29
C. pounduella DSDEKKPLAA KVQPNGSASR DDPMKSASL SNKRPPCEVX SLNGSSLKKP KLSPTTEIS NKGAAVKEEKAEDDDDN Ophinia 50, 355 DEKKPLAA KVQONGSASR DOPMKSASL SNKRPPCEVX SLNGSSLKKP KLSPTTEIS NKGAAVKEE	O. trichocarpon	DSDEKKPLAA	KVQPNGSASR	DSPMNKSASL	SNKRPPGEVK	SLNQSSLKKP	KLSSPITSIS	NKQAAVKPEP	KAEDDDDN	29
Ophiomiza sp. 35 D3DEKKPLAA KVOONGASAR DOPMIKSASL SINKRAPEEVK SLIVOSSLKKY KLSPITISI KKOAAVKPEPKAEDDDDN O. Jukkiensis D3DEKKPLAA KVOONGASAR DOPMIKSASL JINKRAPEEVK SLIVOSSLKKY KLSPITISI KKOAAVKPEP	O. pedunculata	DSDEKKPLAA	KVOPNGSASR	DSPMNKSASL	SNKRPPGEVK	SLNQSSLKKP	KLSSPITSIS	NKQAAVKPEP	KAEDDDDN	29
O. harrisiana DSDEKKPLAR KVOQNOSASE OCPNIKSASEL SNKRAPGEVK SLIOSSLKKP KLSSPTISIS NKOAAVYPEP	Ophiorrhiza sp. 35	DSDEKKPLAA	KVOONGSASR	DGPMNKSASL	SNKRAPGEVK	SLNQSSLKKP	KLSSPITSIS	NKQAAVKPEP	KAEDDDDN	29
O. Ilukiensis babekkplar kvognosase Dopwinsasl LinkapGetvk sluosslukka klsspitsis NKoaAvKPEP	O. harrisiana	DSDEKKPLAA	KVQQNGSASR	DGPMNKSASL	SNKRAPGEVK	SLNQSSLKKP	KLSSPITSIS	NKQAAVKPEP	KAEDDDDN	29
C. D. Delamida DSDEKKPLAA KVOORGAAF GEMINKASL SINKAPGEVK SLNOSSLKKP KLSSTITIS INGAAVKPFKAEDDDDN Comptotheca acuminad DSDEKKPLAA KVOORGAAF DGEMINKASL SINKAPGEVK SLNOSSLKKP KLSSTITIS INGAAVKPF	O. liukiuensis	DSDEKKPLAT	KVQQNGSASR	DGPMNKSASL	LNKRAPGEVK	SLNQSSLKKA	KLSSPITSIS	NKQAAVKPEP	KAEDDDDN	29
Ó fucosa bsoektycza kwoda szak odejmiksze swikaroczy siloszkie kiszkej tals nicazyjepkłebodo Campiotheca acuminata ESDDIKTLYS NFOORGING GSKSIK YNKRPLGEVK SYOSSYKKR KLSDASTYN NIKOASKKAEFKADOSD- Homo sapiens O, pseudofasiculata i pisorgkks GAAISKKOE DODNY I AGR IKKSPSSKS SSAFYKKAST VYSSIKKWK KISKKVI KKT AYTKSSKYP O, pseudofasiculata i pisorgkks GAAISKKOE DODNY I AGR IKKSPSSKS SSAFYKKAST VYSSIKKWK KISKKVI KKT AYTKSSKYP O, pedunculata i pisorgkks GTAISKPKOE DODNY I GR IKKSPSSKS SSAFYKKAST VYSSIKKWK KISKKVI KKT AYTKSSKYP O, piumbe i pisorgkks GTAISKPKOE DODNY I GR IKKSPSSKS SSAFYKKAST VYSSIKKWK KISKKVI KKT AYTKSSKYP O, piumbe i pisorgkks GTAISKPKOE DODNAP I SGR IKKSPSSKS SSAFYKKAST VYSSILKKVI KKT AYTKSSKYP O, Diartisian I pisorgkks GTAISKPKOE DODNAP I SGR I KKSPSSKS SSAFYKKAST VYSSILKKVI KKT AYTKSSKYP O, Diartisian I pisorgkks GTAISKPKOE DODNAP I SGR I KKSPSSKS SSAPYKKAST VYSSILKKVI KKT AYTKSSKYP O, function I pisorgkks GTAISKPKOE DODNAP I SGR I KKSPSSKS SSAPYKKAST VYSSILKKVI KKT AYTKSSKYP O, functionis I pisorgkks GTAISKPKOE DODNAP I SGR I KKSPSSKS SSAPYKKAST VYSSILKKVI KKT AYTKSSKYP O, functionis I pisorgkks GTAISKPKOE DODNAP I SGR I KKSPSSKS SSAPYKKAST VYSSILKKVI KKT AYTKSSKYP O, functionis I pisorgkks GTAISKPKOE DODNAP I SGR I KKSPSSKS SSAPYKKAST VYSSILKKVI KKSKVI KKT AYTKSSKYP C, functionis I pisorgkks GTAISKPKOE DODNAP I SGR I KKSPSSKS SSAPYKKAST VYSSILKKVI KKSKVI KKT AYTKSSKYP C, functionis I pisorgkks GTAISKPKOE DODNAP I SGR I KKSPSSKS SSAPYKKAST VYSSILKKVI KKSKVI KKT AYTKSSKYP Gampiotheca acuminati C, functionaria C, func	O. nuleyana O numila	DSDEKKPLAA	KVQQNGSASP	EGPMNKSASL	SNKRAPGEVK	SLNQSSLKKP	KLSSPITSIS	NKQAAVKPEP	KAEDDDDN	29
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Homo sapiens Homo sapiens<	Camptotheca acuminata	ESDDNKTLVS	NFQQNGSINR	G SKSS1K	VNKRPLGEVK	SSVQSSVKKP	KLSDASTPVN	NKQASKKAEP	KADDSDD -	28
Homo sapiensGKLKKPKIK DKDKKVPEPDNKKKVP KKEEE-OKW KWWEEERYF Catharanthus rosus			340		360		380		400	
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O. frichocarpon O. peduracidal PLSGRCKKS GTATSKPROE DDDNP1SGR O. peduracidal PLSGRCKKS GTAASKPROE DDDNP1SGR KKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. finitiaen, S. PLSGRCKKS GTAASKPROE DDDNP1SGR O. finitiaen, S. PLSGRCKKS GTAASKPROE DDDNP1SGR IKKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. finitiaen, S. PLSGRCKKS GTAASKPROE DDDNP1SGR IKKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. finitiaen, S. PLSGRCKKS GTAASKPROE DDDNP1SGR IKKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. fidleyana IPLSGRCKKS GTAASKPROE DDDNP1SGR IKKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. fidleyana IPLSGRCKKS GTAASKPROE DDDNAP1SGR IKKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. fuccsa IPLSGRCKKS GTAASKPROE DDDNAP1SGR IKKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. fuccsa IPLSGRCKKS GTAASKPROE DDDNAP1SGR IKKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. fuccsa Gamptotheca acuminata IPLSGRCKKS GTAASKPROE DDDNAP1SGR IKKSPASVSK SSAPVKKAST VVSPSLKKVN KKSKKVI KKT AYTKSSKVPP O. ficocarpon GSGEGGWTT LVHNGVIFPP PVRP-HOVK MLYKGPTLL TPEGEVATM FAVMLDTDVM KKPFKENFF SDWKKLI0 O. ficocarpon GSGEGGWTT LVHNGVIFPP PVRP-HOVK MLYKGPTLL TPEGEVATM FAVMLDTDVM KKPFKENFF SDWKKNL0 O. fidliciansis GSGEGGWTT LVHNGVIFPP PVKP-HOVK MLYKGPTLL TPEGEVATM FAVMLDTDVM KKPFKENFF SDWKKNL0 O. fidliciansis GSGEGGWTT LVHNGVIFPP P	O pseudofasciculata	I PL SORGKKS	QAATSKPKDE	DDDNVPIAQR	IKKSPASVSK	SSAPVKKAST	VVSPSLKKVN	KNSKKVIKKT	AYTKSSKVPP	37
0. pedunculata PLSDRGKKS OTAASKYRDE DDDNPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, plumba PLSDRGKKS OTAASKYRDE DDDNAPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, harrisian PLSDRGKKS OTAASKYRDE DDDNAPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, liuklivensis PLSDRGKKS OTAASKYRDE DDDNAPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, liuklivensis PLSDRGKKS OTAASKYRDE DDDNAPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, liuklivensis PLSDRGKKS OTAASKYRDE DDDNAPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, junila PLSDRGKKS OTAASKYRDE DDDNAPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, junila PLSDRGKKS OTAASKYRDE DDDNAPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, junila PLSDRGKKS OTAASKYRDE DDDNAPISOR IKKSPASYSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O, junila HOMO Sapiens - EGI INWFF LEHKGVFAP PYEPLPENVK FYYDGKVMKL SPKAEVATF FAKMLDHEYT TKEIFRKHFF KDWRKKILL-G O, japonica GSEGGGWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFFKENFF SDWKKNLG O, japonica GSEGGGWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFFKENFF SDWKKNLG O, junida GSEGGGWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFFKENFF GSGGGWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFFKENFF SDWKKNLG O, junida GSEGGGWTT LVHNG	O. trichocarpon	IPLSORGKKS	QTATSKPKDE	DDDNVPISOR	IKKSPASVSK	SSAPVKKAST	VVSPSLKKVN	KKSKKVIKKT	AYTKSSKVPP	37
O, piumpea i plsorgkks otaaskprkoe DDDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O. harrisiana i plsorgkks otaaskprkoe DDDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O. ilukiuenisis i plsorgkks otaaskprkoe DDDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O. idiolayana i plsorgkks otaaskprkoe DDDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O. jubili i plsorgkks otaaskprkoe DDDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O. jubili i plsorgkks otaaskprkoe DDDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O. jubili i plsorgkks otaaskprkoe DDDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP O. jubili i plsorgkks otaaskprkoe DDDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP ODDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVIKKT AYTKSSKVPP ODDNAPISGR I KKSPA3VSK SSAPVKKAST VVSPSLKKVN KKSKKVKSK SYKSSKVP Horo sagiens - EG I KWKF LEHKGVI FPP PYKP - HOIK MLYKGAPVOL T PEGEEVATM FAVMLDTDYM NKPFKENFF GDWKKIL- G O. jagonica SSEGGKKWNT LUHKGVI FPP PYKP - HOIK MLYKGAPVIL T PEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jeduncilad SSEGGKWNT LUHKGVI FPP PYKP - HOIK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jeduncilad SSEGGKWTT LUHKGVI FPP PYKP - HOIK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jubinbee SSEGGKWNT LUHKGVI FPP PYKP - HOIK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jubinbee SSEGGKWNT LUHKGVI FPP PYKP - HOIK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jubinbee SSEGGKWNT LUHKGVI FPP PYKP - HOIK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jubinbee SSEGGKWNT LUHKGVI FPP PYKP - HOIK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jubinbee SSEGGKWNT LUHKGVMFPP PYKP - HOIK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jubinbee SSEGGKWNT LUHKGVMFPP PYKP - HOIK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKIL- G O. jubinbee SSEGGKWNT LUHKGVMFPP PYKP - HOKK MLYKGAPVIL TPEGEEVATM FAVMLDTDYM NKPFKF	O. pedunculata	IPLSQRGKKS	QTATSKPKDE	DDDNVPISQR	IKKSPASVSK	SSAPVKKAST	VVSPSLKKVN	KKSKKVIKKT	AYTKSSKVPP	37
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O. pedunculata GSGEGGKWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEOEEVATM FAVMLDTDYM NKPRFKENFF SDWKKMLG O. plumbee GSGEGGKWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEOEEVATM FAVMLDTDYM NKPRFKENFF SDWKKMLG O. harrisiana GSGEGGKWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEOEEVATM FAVMLDTDYM NKPRFKENFF SDWKKMLG O. harrisiana GSGEGGKWTT LVHNGVMFLP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAVMLDTDYM TKPRFKENFF SDWKKMLG O. liukiuensis GSGEGGKWTT LVHNGVMFLP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAVMLDTDYM TKPRFKENFF SDWKKMLG O. ridleyana GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAVMLDTDYM TKPRFKENFF SDWKKMLG O. ridleyana GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAVMLDTDYM NKPRFKENFF SDWKKILG O. ridleyana GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucias GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucias GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucias GSGEGGKWTT LVHNGVIFPP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucias GSGEGGKWTT LVHNGVIFPP PYKPHGVK MLYKCOPLTL TPEOEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucias GSGEGGKWTT LVHNGVIFPP PYKP-HGVK MLYKCOPLT TPEOEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucias GSGEGGKWTT LVHNGVIFPP PYKP-HGVK MLYKCOPLT TPEOEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucias GSGEGGKWTT LVHNGVIFPP QKKHGVK MLYKCOPLT TPEOEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucias GSGEGGKWT LVHNGVIFPP QKKHGVK MLYKGKPVLT TEEKKALKE EKLOGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pseudofasciulata KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE EKLOGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. fucias KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE EKLOGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. fucias KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE EKLOGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. fucias KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. fucias KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTE	O. pseudolasciculata	GSGEGQKWTT	LVHNGVIEPP	PYKP HGVK	MLYKROPITL	TPEQEEVATM	FAVMLDTDYM	NKPCFKENFF	SDWKKILG	450 451
O. plumbea GSGEGGKWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFFKENFF SDWKKMLG Ophiorfhiza sp. 35 GSGEGGKWTT LVHNGVIFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFFKENFF SDWKKMLG O. harrisiana GSGEGGKWTT LVHNGVMFLP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFFKENFF SDWKKMLG O. indivana GSGEGGKWTT LVHNGVMFLP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFFKENFF SDWKKMLG O. indivana GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKMLG O. pumila GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAVMLDTDYM NKPFKENFF SDWKKILG O. fucosa GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAAMLDTDYM NKPFKENFF SDWKKILG O. fucosa GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAAMLDTDYM NKPFKENFF SDWKKILG O. fucosa GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKROPITL TPEGEEVATM FAAMLDTDYM NKPFKENFF SDWKKILG O. fucosa GSGEGGKWTT LVHNGVIPP PYKPHGVK MLYKGPLTL TPEGEEVATM FAAMLDTDYM NKPFKENFF SDWKKILG G Homo sapiens EKNITNLSK CDFTOMSOVF KAGTEAKKOM TTEEKKALKE ENEKLLKEVG FGIMDNHKER IANFKIENFF Gatharanthus roseus KNHVIONLEN CDFSPIYEWH QSEKEKKKOM TTEEKKALKE EKLKGEEKYM WAWVDGKKEK VGNFRVEPPG LFRGRGEHPK O. japonica KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pseudofasciculat KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. plumbea KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. plumbaa KNHTIONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. harrisiana KNHTVONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. harrisiana KNHTVONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. indrisiana KNHTVONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. indrisiana KNHTVONLED CDFGPIYEWH QGEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. indrisiana KNHTVONLED CDFGPIYEWH QGEKEKK	O. pedunculata	GSGEGQKWTT	LVHNGVIFPP	PYKP HGVK	MLYKROPITL	TPEQEEVATM	FAVMLDTDYM	NKPRFKENFF	SDWKKML G	45
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O. pumila GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKROPLTL TPEGEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG O. fucosa GSGEGGKWTT LVHNGVMFPP PYKPHGVK MLYKROPLTL TPEGEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG Camptotheca acuminata SSGEGKKWNT LVHNGVIFPP PYKPHGVK MLYKGKPVDL TPEGEEVATM FAAMLDTDYM NKPRFKENFF DDWRKILG Momo sapiens EKNIITNLSK CDFTOMSOYF KAQTEARKOM SKEEKLKIKE ENEKLLKEYG FGIMDNHKER IANFKIEPPG LFRGRGNHPK Catharanthus roseus KNHVIONLEN CDFSPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. japonica KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pseudofasciculata KNHMIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pedunculata KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pedunculata KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pedunculata KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. piumbaa KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. juumbaa KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. iukiuensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. iukiuensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. iukiuensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. iukiuensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. iukiuensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. juumila KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. juumila KNHTIONLED CDFGPIYEWH QSEKEKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. juumila KNHTIONLED CDFGPIYEWH QSEKEKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. juuxila	O. ridleyana	GSGEGQKWTT	LVHNGVMFPP	PYKP HGVK	MLYKRQPLTL	TPEQEEVATM	FAVMLDTDYM	NKPRFKENFF	SDWKKILG	45
O. Jucosa csgeggkwitt LVHNgVIFPP PYKPHGVK MLYKGPLTL TPEQEEVATM FAAMLDTDYM NKPRFKENFF SDWKKILG Camptotheca acuminata Ssgegkwitt LVHNgVIFPP PYKPHGVK MLYKGPVDL TPEQEEVATM FAAMLDTDYM TKSKFKENFM DDWRLIG 500 540 540 540 540 540 540 540 540 540	O. pumila	GSGEGQKWTT	LVHNGVMFPP	PYKP HGVK	MLYKROPLTL	TPEQEEVATM	FAAMLDTDYM	NKPRFKENFF	SDWKK L G	45
Homo sapiens EKNIITNLSK CDFTQMSQYF KAQTEARKOM SKEEKLKIKE IEKKLLKEYG FCIMDNHKER IANFKIEPPG LFRGRGEHPK OCATABATATIONLED CDFSPIYEWH QSEKEKKKOM TTEEKKALKE EKLKQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. japonica knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jedunculata knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jedunculata knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jedunculata knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jedunculata knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jedunculata knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jedunculata knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jumbea knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jiukiuensis knhtvonled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jiukiuensis knhtvonled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jiukiuensis knhtvonled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. jumila knhtionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. juckiem antionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. juckiem antionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. juckiem antionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. juckiem antionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. juckiem antionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. juckiem antionled cdfspiyewh qoekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. juckiem antionled cdfspiyewh qoeke	O. tucosa Camptotheca acuminata	GSGEGQKWTT		PYKP HGVK	MLYKROPLTL	TREGEEVATM	FAAMLDTDYM	NKPRFKENFF	SDWKKILG	451 404
Homo sapiens EKNIITNLSK CDFTOMSOYF KAQTEARKOM SKEEKLKIKE ENEKLLKEYG FCIMDNHKER IANFKIEPPG LFRGRGHPK Catharanthus roseus Knhvionlen Cdfspiyewh osekekkkom tteekkalke eklkoeekym wavvdgkkek vgnfrveppg Lfrgrgehpk O. japonica knhtionled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. beudofasciculata knhmionled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. trichocarpon knhtionled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. trichocarpon knhtionled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. pedunculata knhtionled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. plumbea knhtionled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. harrisiana knhtvonled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. harrisiana knhtvonled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. indivensis knhtvonled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. indivensis knhtvonled cdfspiyewh ogekekkkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. indivensis knhtvonled cdfspiyewh ogekekrkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. indivensis knhtvonled cdfspiyewh ogekekrkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. judmila knhtionled cdfspiyewh ogekekrkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. pumila knhtionled cdfspiyewh ogekekrkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. pumila knhtionled cdfspiyewh ogekekrkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. pumila knhtionled cdfspiyewh ogekekrkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk O. pusikek vgnfrveppg Lfrgrgehpk ogekekrkom tteekkalke ekloqeekym waivdgvkek vgnfrveppg Lfrgrgehpk D. putpychow bekekpygehpkehpkehpkehpkehpkehpkehpkehpkehpkehpk	Samplotnova avunnilala	- JOE GRAWN I	500		520	TREGEEVAIM	540	INGNENENEM	560	+£\
Catharanthus roseus KNHVIONLEN COFSPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAWVDGKKEK VGNFRVEPPG LFRGRGEHPK O. japonica KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. trichocarpon KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. trichocarpon KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pedunculata KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pedunculata KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pedunculata KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE EKLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. phiornhiza sp. 35 KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. harrisiana KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. indievensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. indievensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. indievensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. indievensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. indievensis KNHTVONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QSEKEKKKOM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. puckek VGNFRVEPPG LFRGRGEHPK OM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK D. puckek VGNFRVEPPG LFRGRGEHPK OM TTEEKKALKE ERLQGEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK	Homo saniens		CDETONSOVE	KAOTEAPKON	SKEEKIKIKE					364
O. japonica knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE DKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pseudofasciculata knhmionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. trichocarpon knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. podunculata knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. piumbea knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. piumbea knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. harrisiana knhtvonled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. liukiuensis knhtvonled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. liukiuensis knhtvonled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. ridibyana knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQUEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK D. pumila knhtionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQUEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK	Catharanthus roseus	KNHVIONLEN	CDFSPIYEWH	OSEKEKKKOM	TTEEKKALKE	EKLKQEEKYM	WAWVDGKKEK	VGNFRVEPPG	LFRGRGEHPK	008 514
O. pseudotasciculata KNHMIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. trichocarpon KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pedunculata KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. plumbea KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. plumbea KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. harrisiana KNHTVONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM KAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. liukiuensis KNHTVONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. ridleyana KNHTVONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. ridleyana KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK D. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK D. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQUEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK D. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQUEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK	O. japonica	KNHTIQNLED	CDFGPIYEWH	QQEKEKKKQM	TTEEKKALKE	DKLQQEEKYM	WAIVDGVKEK	VGNFRVEPPG	LFRGRGEHPK	532
 O. BIGLIOGALPUT KNHTIONLED CDFGPIYEWH QOEKEKKKOM TTEEKKALKE EKLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK I O. pedunculata knhtionled cdfgpiyewh qoekekkkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. plumbea knhtionled cdfgpiyewh qoekekkkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i Ophiomtiza sp. 35 knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. harrisiana knhtvonled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Raivdgvkek vgnfrveppg Lfrgrgehpk i O. harrisiana knhtvonled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. indibyana knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. indibyana knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. juumila knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. pumila knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. pumila knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. pumila knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqqeekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. pumila knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqleekym Waivdgvkek vgnfrveppg Lfrgrgehpk i O. pugnila knhtionled cdfgpiyewh qoekekrkom tteekkalke eklqleekym Waivdgvkek vgnfrveppg Lfrgrgehpk i 	O. pseudofasciculata	KNHMIQNLED	CDFGPIYEWH	QQEKEKKKQM	TTEEKKALKE	EKLQQEEKYM	WAIVDGVKEK	VGNFRVEPPG	LFRGRGEHPK	530
O. plumbea KNHTIONLED CDFGPIYEWH QQEREKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK Ophioriniza sp. 35 KNHTIONLED CDFGPIYEWH QQEREKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. harrisiana KNHTVONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. liukiuensis KNHTVONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. ridleyana KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQUEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. pumila KNHTIONLED CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQUEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK	O. uncriocarpon	KNHTIONLED	CDEGPIYEWH	QOEKEKKKOM	TTEEKKALKE	EKLOQEEKYM	WAIVDGVKEK	VGNERVEPPG	LERGROENER	531 521
Ophiomhiza sp. 35 knhtionled CdFGPiyewh qqerekkkom tteekkalkd erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. harrisiana knhtvonled CdFGPiyewh qqekekrkom tteekkalke erloqeekym raivdgvkek vgnfrveppg lfrgrgehpk O. liukiuensis knhtvonled CdFGPiyewh qqekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. ridleyana knhtionled CdFGPiyewh qqekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. pumila knhtionled CdFGPiyewh qqekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. pumila knhtionled CdFGPiyewh qqekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. pumila knhtionled CdFGPiyewh qqekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk D. gucsa knhtionled CdFGPIyewh qqekekrkom tteekkalkb erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk	O. plumbea	KNHTIQNLED	CDFGPIYEWH	QQEKEKKKOM	TTEEKKALKE	EKLOQEEKYM	WVIVDGVKEK	VGNFRVEPPG	LFRGRGEHPK	53
O. narrisiana knhtvonled cdfgpiyewh qoekekrkom tteekkalke erloqeekym Raivdgvkek vgnfrveppg lfrgrgehpk O. liukiuensis knhtvonled cdfgpiyewh qoekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. ridieyana knhtionled cdfgpiyewh qoekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. pumila knhtionled cdfgpiyewh qoekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. pumila knhtionled cdfgpiyewh qoekekrkom tteekkalke erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. gumila knhtionled cdfgpiyewh qoekekrkom tteekkalkd erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk D. gumila knhtionled cdfgpiyewh qoerekrkom tteekkalkd erloqeekym waivdgvkek vgnfrveppg lfrgrgehpk	Ophiorrhiza sp. 35	KNHTIQNLED	CDFGPIYEWH	QQEREKKKQM	TTEEKKALKD	ERLQQEEKYM	WAIVDGVKEK	VGNFRVEPPG	LFRGRGEHPK	53
O. MUNUEMINS KNHTYUNLED CDFGPITEWH QQEKEKRKOM TTEEKKALKE ERLQQEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. midleyana knhtionled cdfgpiyewh qqekekkkom tteekkalke erlqqeekym waivdgvkek vgnfrveppg lfrgrgehpk O. pumila knhtionled cdfgpiyewh qqekekkkom tteekkalke erlqleekym waivdgvkek vgnfrveppg lfrgrgehpk O. fucosa knhtionled cdfgpiyewh qqekekkkom tteekkalkd erlqleekym waivdgvkek vgnfrveppg lfrgrgehpk	O. harrisiana	KNHTVQNLED	CDFGPIYEWH	QQEKEKRKQM	TTEEKKALKE	ERLOQEEKYM	RAIVDGVKEK	VGNFRVEPPG	LFRGRGEHPK	53
O. pumila knitionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKE ERLQLEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK O. fucosa knitionled CDFGPIYEWH QQEKEKKKOM TTEEKKALKD ERLQLEEKYM WAIVDGVKEK VGNFRVEPPG LFRGRGEHPK	O. IIUKIUENSIS O ridlevana	KNHTIONLED	CDEGPIYEWH	QUEKEKKKOM	TTEEKKALKE	ERLOQEEKYM	WAIVDGVKEK	VGNERVEPPG	LERGROEHPK	531
O JUCOSA KNHTIONLED COFGPIYEWH QOEREKKKOM TTEEKKALKD ERLOLEEKYM WAIVDGVKEK VONFRVEPPG LFRGRGEHPK	O. pumila	KNHTIQNLED	CDFGPIYEWH	QQEKEKKKOM	TTEEKKALKE	ERLOLEEKYM	WAIVDGVKEK	VGNFRVEPPG	LFRGRGEHPK	531
Comptethees couminate way used as a series of the series o	O. fucosa	KNHTIQNLED	CDFGPIYEWH	QQEREKKKQM	TTEEKKALKD	ERLQLEEKYM	WAIVDGVKEK	VGNFRVEPPG	LFRGRGEHPK	53

		580		600		620		640
Homo sapiens	MGMLKRRIMP	EDILINCSKD	AKVPS-PPPG	HKWKEVRHDN	KV TWL VSWTE	NI-QGSIKYI	MLNPSSRIKG	EKDWQKYETA 447
Catharanthus roseus	MGKLKKRIRP	CDITINIGKD	APIPECPVPG	ERWKEVRHDN	TVTWLAFWND	PINPKEFKYV	FLAASSTLKG	LSDKEKYEKA 594
O. pseudofasciculata	MGKLKKRIRP	RDITINIGKD	APIPECPIPG	ERWKEVRNON	TVTWLAYWND	PINGKEFKYV	FLAASSILKG	QSDKEKYEKA 612
O. trichocarpon	MGKLKKRIRP	RDITINIGKD	APIPECPIPG	ERWKEVRNDN	TVTWLAYWND	PINQKEFKYV	FLAASSTLKG	QSDKEKYEKA 611
O. pedunculata	MGKLKKRIRP	RDITINIGKD	APIPECPIPG	ERWKEVRNDN	TVTWLAYWND	PINQKEFKYV	FLAASSTLKG	QSDKEKYEKA 611
Ophiorrhiza sp. 35	VGKLKKRIKP	RDITINIGKD	APIPECPIPG	ERWKEVRNDN	TVTWLAYWND	PUNLKECKYV	FLGASSILKG	OSDKEKYEKA 611
O. harrisiana	VGKLKKRIHP	RDITINIGKD	APIPECPIPG	ERWKEVRNDN	TVTWLAYWND	PVNLKECKYV	FLAPSSTLKG	QSDKEKYEKA 611
O. liukiuensis O. ridlevana	MGKLKKRIRP	RDITINIGKD	APIPECPIPG	ERWKEVRNDN	TVTWLAFWND	PINQKECKYV	FLAASSTLKG	QSDKEKYEKA 611
O. pumila	VGKLKKRIRP	RDITINIGKD	APIPECPIPG	ERWKEVRNDN	TVTWLAYWND	PVNLKECKYV	FLAASSTLKG	QSDKEKYEKA 611
O. fucosa	VGKLKKRIHP	RDITINIGKD	APIPECPIPG	ERWKEVRNDN	TVTWLAYWND	PVNLKECKYV	FLAPSSTLKG	QSDKEKYEKA 611
Campioineca acuminaia	MGKLKKCIRP	SDITINIGKD	APIPECPIPG	ESWKEIRHDN	TVTWLAFWND	PIKPREFKYV	FLAASS <mark>S</mark> LKG	QSDKEKYEKA 580
Homo saniens						ETADTV0000		
Catharanthus roseus	RLLKDYIQGI	RAAYTKDFAS	-KDPTKKQIA	VATYLIDKLA	LRAGNEKDDD	E-ADTVGCCT	LKVENVEPVP	PNILK 667
O. japonica	RLLKDYIHGI	RAAYTKDFTN	NKDPMKKQIA	VATYLIDKLA	LRAGNEKDDD	E-ADTVGCCT	LKVENVEPVP	P NILK 686
O. pseudofasciculata	RLLKDYIHGI	RAAYTKDETN	NKDPMKKQIA	VATYLIDKLA		E-ADTVGCCT	LKVENVEPVP	P NILK 684
O. pedunculata	RLLKDYIHGI	RAAYTKDFTN	NKDPMKKQIA	VATYLIDKLA	LRAGNEKDDD	E-ADTVGCCT	LKVENVEPVP	P NILK 685
O. plumbea	RLLKDYIHGI	RAAYTKDFTN	NKDPMKKQIA	VATYLIDKLA	LRAGNEKDDD	E-ADTVGCCT	LKVENVEPVP	P NILK 685
Opniormiza sp. 35 O harrisiana	RLLKDYIHGI	RAAYTKDETN	NKDPMKKQIA	VATYLIDKLA		E-ADTVGCCT		PNILK 685
O. liukiuensis	RLLKDYIHGI	RAAYTKDETN	NKDPMKKQIA	VATYLIDKLA	LRAGNEKDDD	E-ADTVGCCT	LKVENVEPVP	PNILK 685
O. ridleyana	RVLKDYIHGI	RAAYTKGFTN	NKDPMKKQIA	VATYLIDKLA	LRAGNEKDDD	E-ADTVGCCT	LKVENVEPVP	P NILK 685
O. pumila O fucosa	RLLKDYIHGI	RAAYTKDETN	NKDPMKKQVA	VATYLIDKLA		E-ADTVGCCT	LKVENVEPVP	PNILK 685
Camptotheca acuminata	RLLKDFIQGI	RAAYTKDFAS	- KDITKRQIA	VATYLIDKLA	LRAGNEKDDD	E-ADTVGCCT	LKVENVEPKP	PSILK 653
		740		760		780		800
Homo sapiens	FDFLGKDSIR	YYNKVPVEKR	VEKNLQLEME	NKOPEDDLFD	RLNTGILNKH	LQDLMEGLTA	KVFRTYNASI	TLQQQLKELT 606
Catharanthus roseus	FDFLGKDSIR	YQNEVEVEAA	VFKAIQQFRS	GKEGSSDLFD	LLDTSKLNAH	LKELMPGLTA	KVFRTYNASI	TLDDMLSRET 747
O. pseudofasciculata	FDFLGKDSIR	YONEVQVEPA	VEKALQOERS	GKEGSEDLFD	RLDTSKLNAH	LKELMPGLTA	KVFRIYNASI	TLDDMLSKET 766
O. trichocarpon	FDFLGKDSIR	YQNEVQVEPA	VFKAIQQFRS	GKEGSEDLFD	RLDTSKLNAH	LKELMPGLTA	KVFRTYNASI	TLDDMLSKKT 765
O. pedunculata	FDFLGKDSIR	YQNEVQVEPA	VFKAIHQFRS	GKEGSEDLFD	RLDTSKLNAH	LKELMPGLTA	KVFRTYNASI	TLDDMLSKKT 765
Ophiorrhiza sp. 35	DEIGKDSIR	YONEVOVEPA	VEKALQOERS	GKEGSEDLED	RLDTSKLNAH	LKELMPGLTA	KVFRTYNASI	TLDDMLSKET 765
O. harrisiana	DFIGKDSIR	YQNEVQVEPA	VFKAIQQFRS	GKEGSEDLFD	RLDTSKLNAH	LKELMPGLTA	KVFRTYNASI	TLDDMLSKET 765
O. liukiuensis O. ridlevana	DFIGKDSIR	YQNEVQVEPA	VFKAIQQFRS	GKEGCADLFD	RLDTSKLNAH	LKELMPGLTA	KVFRTYNASI	TLDDMLSKET 765
O. pumila	DFIGKDSIR	YQNEVQVEPA	VEKALQOERS	GKEGSEDLFD	RLDTSKLNAH	LKELMPGLTA	KVFRTYNASI	TLDDMLSKET 765
O. fucosa	DFIGKDSIR	YQNEVQVEPA	VFKAIQQFRS	GKEGSEDLFD	RLDTSKLNAH	LKELMPGLTA	KVFRTYNASI	TLDDMLSKET 765
Camptotneca acuminata	FDFLGKDSIR	YONEVEVELP	VYKALOOFRT	GKRGGDDLFD	KLDTSKLNAH	LKGLMPGLTA	KVFRTYNASI	TLDEMLSRET 733
		1		1				
Catharanthus roseus	KGGD-VAEKV	VYYOHANKEV	ALICNHORTV	SKSHSAOMLR	LNEKIEELKA	VVEELKSDLS	RVKKGKPPLK	SKNADGKPKR 826
O. japonica	KGGE - VAEKV	VVYQHANKEV	AIICNHQRTV	SKSHSAQMSR	LNEKIDELKT	AMEELKTDLS	RVKKGKPP	SKGADGKPKR 843
O. pseudofasciculata	KGGE - VAENV	VVYQHANKEV	AIICNHQRTV	SKSHSAQMSR	LNEKIDELKT	ALEELKTDLS	RAKKGKPP	TKGADGKAKR 841
O. Inchocarpon O. pedunculata	NGGE - VAEKV	VYYQHANKEV	ALICNHORTV	SKSHSAQMSR	LNEKIDELKT	ALEELKIDLS	RAKKGKPL	SKGADGKPKR 842
O. plumbea	KGGK - VAEKV	GVYQHANKEV	AIICNHQRTV	SKSHSAQMSR	LNEKIDELKT	ALEELKTDLS	RAKKGKPP	SKGADGEPKR 842
Ophiorrhiza sp. 35	KGGK - VAEKV	GVYQHANKEV	ALICNHORTV	SKSHSAQMSR	LNEKIDELKT	ALEELKTDLS	RAKKGKPP	SKGDDGEPKR 842
O. liukiuensis	KGGK - VAEKV	GVYQHANKEV	ALICNHORTV	SKSHSAQMSR	LNEKIDELKT	ALEELKTDLS	RAKKGKPP	AKGADGEPKR 842
O. ridleyana	KGGK- AEKV	AVYQHANKEV	AIICNHQRTV	SKSHSAQMSR	LNEKIDELKT	ALEELKTDLS	RAKKGKPP	SKDADGEPKR 842
O. pumila O. fucosa	KGGK - VAEKV	GVYQRANKEV	ALICNHORTV	SKSHSAQMSR	LNEKIDELKT	ALEELKTDLS	RAKKGKPP	SKGDDGEPKR 842
Camptotheca acuminata	KGGN - VAEKI	VVYQHANKEV	AIICNHORTV	SKSHPAQMTR	LNGKIDELKG	ILDGLQTDLA	RAKKGKPPL -	- EDADGKPKR 810
		900		920		940		960
Homo sapiens	VVESKKKA	VQRLEEQLMK	LEVQATDREE	NKQIALGTSK	LNYLDPRITV	AWCKKWGVPI	EKIYNKTORE	KFAWA I DMAD 760
Catharanthus roseus	NLNPESLEKK	IAQTNAKIEK	MERDKETKED	LKTVALGTSK	INYLDPRITV	AWCKRHEVPI	EKIFNKSLLA	KFTWAMDV-D 905
O. japonica O. pseudofasciculata	NLNPEALERK	ITOTNAKIEK	MERDKETKED	LKTVALGTSK	INYLDPRITV	AWCKRHEVPI	EKIFNKSLLA	KFAWSMDV-D 922
O. trichocarpon	NLNPEALERK	IAQTNAKIEK	MERDKETKED	LKTVALGTSK	INYLDPRITV	AWCKRHEVPI	EKIFNKSLLA	KFAWAMN 919
O. pedunculata	NLNPEALERK	IAQTNAKIEK	MERDKETKED	LKTVALGTSK	INYLDPRITV	AWCKRHEVPI	EKIFNKSLLA	KFAWAMN 919
Ophiorrhiza sp. 35	NLNPEALERK		MEGDKKTKED	LKAVALSTSK	ISTLOPRITV	AWCKROEVPI	EKMENKSLLA	KFAWAMN 919
O. harrisiana	NLNPEALERK	IAQTNAKIEK	MERDKKTKED	LKAVALSTSK	ISYLDPRITV	AWCKROEVPI	EKMENKSLLA	KFAWAMN 919
O. liukiuensis	NLNPESLERK	IAQTNAKIEK	MERDKKTKED	LKAVALSTSK	ISYLDPRITV	AWCKRHEVPI	EKIFNKSLLA	KFAWSMDV - D 921
O. pumila	NLNPESLERK	IAQTNAKIEK	MERDKKTKED	LKAVALSTSK	ISYLDPRITV	AWCKROEVPI	EKMENKSLLA	KFAWAMDV-D 921
O. fucosa	NLNPEALERK	IAQTNAKIEK	MERDKKTKED	LKAVALSTSK	ISYLDPRITV	AWCKROEVPI	EKMFNKSLLA	KFAWAMDV-D 921
Campioineca acuminata	NLTPEALERK	GQTNAKIEK	MERDKETKEG	LKTIALGTSK	ISYLDPRITV	AWCKRHEVPI	EKVFNKSLLA	KFAWSMDV-D 889

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Publication

Viraporn, V., Yamazaki, M., Saito, K., Denduangboripant, J., Chayamarit, K., Chuanasa, T., and Sukrong, S. 2011. Correlation of camptothecin-producing ability and phylogenetic relationship in the genus *Ophiorrhiza* (Rubiaceae). <u>Planta medica</u> (accepted).

