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Editor's Note

The year B.E. 2552 (A.D. 2009) has brought about a number of changes in connection with this journal. First, it is now entering its 3rd year of activity since its conception with its first volume and first issue being launched 2 years ago. Second, it is now a 100% e-journal (no more hard copies), which means an article can be published anytime as soon as it is ready (as always being the case from the beginning, however.)

Thirdly, our managing editor, Dr. Weerachai Phutdhawong, the technical and key founder of this journal, has reluctantly left us for a new academic position at Kasetsart University. Without him from the start, this journal would never have been as it is now. He and the webmasters of Maejo University have jointly created a website for a journal which is freely, fully, and easily accessible. And this is most probably one of the factors that contribute to its unexpected and continuing popularity from the beginning as well as to the increasing international recognition of the journal now.*

Lastly, the editor sincerely hopes that, with a well-laid foundation in store and a strong editorial committee at present, and despite his failing health after two years in office, which may result in a new editor for the journal in the near future, this journal will continue on well towards serving submitters, both local and abroad, as well as improving on its standard further.

Duang Buddhasukh
Editor

* In addition to being covered by DOAJ, SciFinder Scholar, Chemical Abstracts and AGRIS, this journal has now been selected for coverage in Thomson Reuters products and custom information services. Beginning with Vol. 1(1), 2007, it will be indexed and abstracted in Science Citation Index Expanded (SciSearch) and Journal Citation Reports/Science Edition.

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Report

Vascular flora of the Emerald Pool area, Krabi province, southern Thailand

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Abstract: The Emerald Pool is situated in remnant lowland (25-75 m), seasonal, fresh water, swamp forest on limestone bedrock. Primary, evergreen, seasonal, hardwood forest, often with bamboo and frequently degraded, surrounds the swamp forest and extends to 175 m elevation. The bedrock above the swamp forest is sandstone with occasional limestone outcrops and hills. A total of 111 vascular plant families with 420 species were found.

Keywords: Emerald Pool, Krabi flora and vegetation

Introduction

The Emerald Pool (Sra Moragote) is located in Khao Pra-Bahng Krahm Wildlife Sanctuary, Klong Tawm Nua subdistrict, Klong Tawm district, Krabi province at approximately 8°N latitude, 99° E longitude. The area is an ecologically distinct habitat in lowland (25-75 m elevation), fresh water, seasonal, swamp forest on limestone bedrock. There are several hot springs, all less than 40°C on limestone bedrock, in the area—the Emerald Pool being the largest and most intact of them.

Primary, evergreen, seasonal, hardwood + bamboo forest, some of it very degraded, on sandstone bedrock is present adjacent to the swamp forest to the summit of Pan Din Samur (*c.* 175 m elevation)—the highest point in the research area.

The sanctuary was established in 1993, mainly to protect the last known populations of Gurney's Pitta (*Pitta gurney*i) in Thailand. This is a ground-living bird that requires intact swamp

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forest for survival. My work there started in late September 2005 with several occasional visits since then. The sanctuary includes an area of 156 km^2 with only *c*. 25 km^2 of forested land.

Background

During the latter half of the 20th century, southern Thailand was largely deforested and much of the land planted with monocultures of *Hevea brasilensis* (Kunth) M.A. (Euphorbiaceae, Para rubber, from South America) and *Elaeis guineensis* Jacq. (Palmae, oil palm, native to tropical west Africa)—both economically valuable trees. After regional communist disturbances were resolved in 1983, many people migrated to Klong Tawm Nua Subdistrict where consequent severe forest encroachment caused drastic reduction in Gurney's Pitta populations. Presently, the entire sanctuary is surrounded and dissected by Para rubber and oil palm plantations while forest destruction continues, although at a slower rate than previously.

In an effort to restore primary forest cover to destroyed areas originally inhabited by Gurney's Pitta, the Royal Society for the Protection of Birds (U.K.) contacted the Forest Restoration Research Unit (FORRU), Chiang Mai University, of which I am a member, to properly replant forests there. To effect this, a detailed study of the flora there was required. Collaborative work with FORRU enabled the reforestation project to begin planting in June 2006.

This area is more widely known internationally as Khao Nor Chuchi, a limestone mountain rising to 650 m elevation, a few kilometers to the south-east of the Emerald Pool.

Conservation

Tina Jolliffe (1947-1993), a British conservationist, established the Children's Tropical Forests (U.K.) in 1989. A fund was developed to improve conservation and education in the sanctuary, and a nature trail around the Emerald Pool was established by 1992 [1,2]. Unfortunately, this 2.7- km- long Tina Jolliffe Nature Trail was not maintained or improved. The Tourist Authority of Thailand built a boardwalk in the swamp forest in 1999 which has been maintained. One of my goals during my work there was to produce list of trees along the nature trail, which now includes nearly 100 species.

Geology

The Geological Map of Thailand [3] indicates that the Emerald Pool area consists of Triassic limestone and sandstone (Lampang Group), which was originally formed from deep sea sediment approximately 200 million years ago and later uplifted. Fresh water limestone (tufa) has developed in the swamp forest, often forming extensive, open, barren areas near the Emerald Pool (Photos 1 and 2).

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Photo 1. Open, moist to wet tufa above the Emerald Pool with scattered patches of vegetation, including *Eugenia papillosa* Duth. (Myrtaceae), shown here, being abundant.

Photo 2. Open tufa bordering the swamp forest with a drainage channel leading to the Emerald Pool.

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Climate

The climate throughout Thailand is seasonal. The provinces bordering Malaysia are the least seasonal and have a dry period of up to 6 weeks, while the northern provinces have a dry period of 4-6 months. As latitude decreases the temperature is less variable resulting in two seasons in the southern provinces, viz. dry and rainy. In northern Thailand there is a wide range of temperature variation which causes three seasons, viz. cool/dry, hot/dry, and rainy.

Krabi province has a distinct dry season from December to March with rains starting in April and peaking in July (Figure 1). The average amount of annual rainfall at Krabi during 1995-2004 ranged from 1796 m to 2387 mm–mean average of *c.* 2000 mm/year (Figure 2).

Vegetation

Fresh water swamp forest

The fresh water swamp forest is a 2.5-km^2 remnant area around the Emerald Pool which is mostly undisturbed and has moist to wet soil throughout the year (Photos 3 and 4). In general, the land is flat, densely vegetated, and at *c*. 25-75 m elevation. Marginal areas vary from destroyed, regenerating, or better drained, primary, evergreen, seasonal, hardwood + bamboo forest on limestone and sandstone bedrock with little tufa.

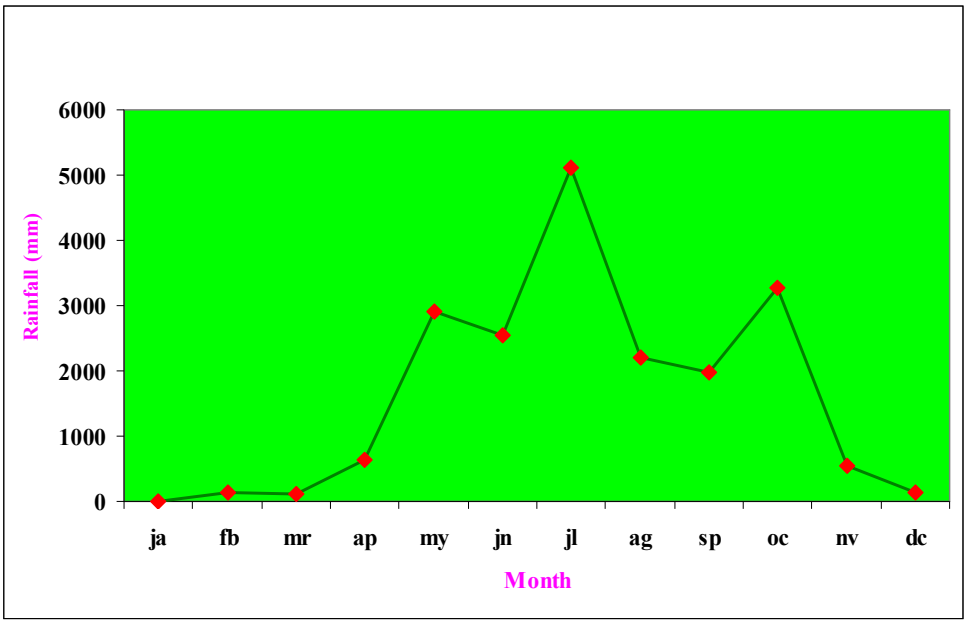

Figure 1. Average monthly rainfall (mm) at Krabi (1995 - 2004) [Source: Krabi Meteorological Station]

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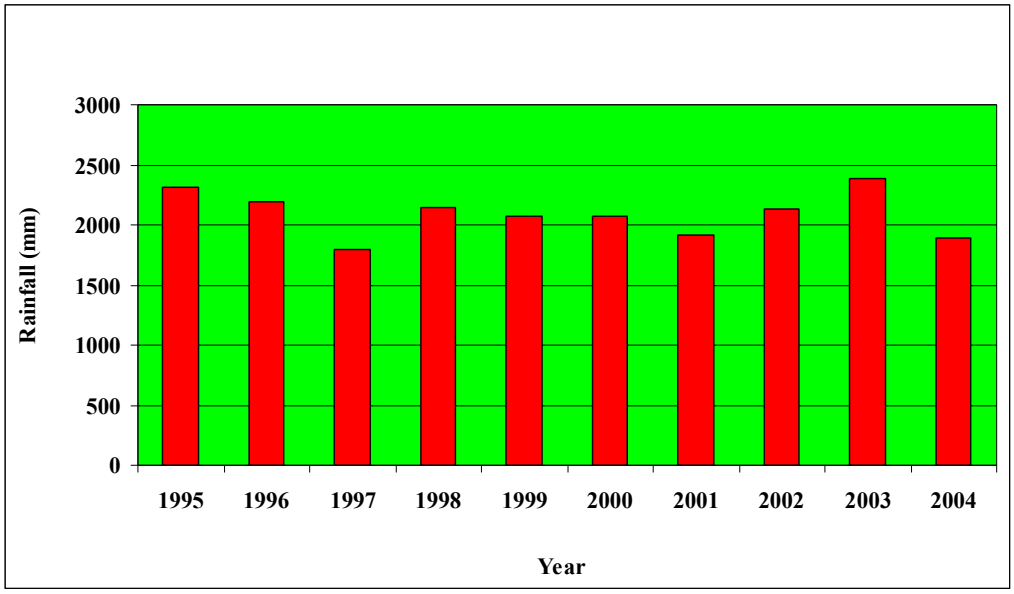

Figure 2. Average annual rainfall (mm) at Krabi (1995 - 2004). Source: Krabi Meteorological Station

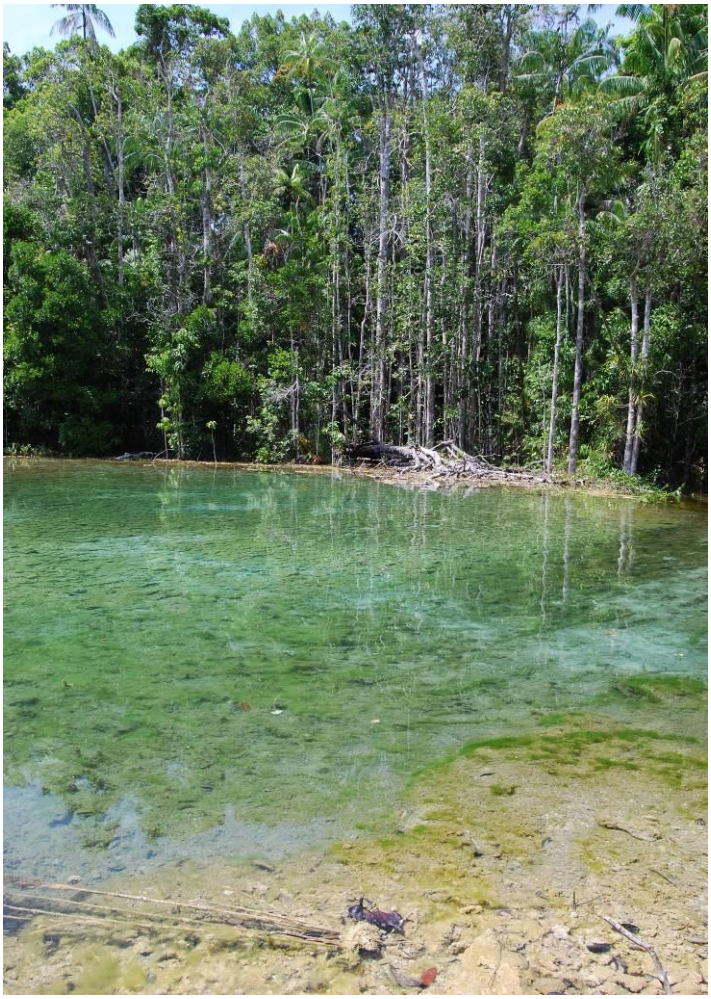

Photo 3. A pool in the swamp forest with calcareous mud and typically warm, blue-green water.

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Photo 4. A typical wet area in the swamp forest where the water lacks warmth and pigmentation.

The ground flora consists of many evergreen herbs dominated by *Dracena curtisii* Ridl. (Agavaceae), *Aglaonema oblongifolium* (Roxb.) Schott (Araceae), *Donax cannaeformis* (G. Forst.) K. Sch. (Marantaceae), *Tacca chantrieri* Andre (Taccaceae), *Globba fasciata* Ridl. (Zingiberaceae), and *Freycinetia sumatrana* Hemsl. var. *sumatrana* (Pandanaceae), a creeping vine ―all monocots. Dicots are relatively sparse with *Acrotrema costatum* Jack (Dilleniaceae), *Sericocalyx glaucescens* (Nees) Brem. (Acanthaceae), *Adenosoma indiana* (Lour.) Merr. (Scrophulariaceae), and *Thottea tomentosa* (Bl.) Hou (Aristolochiaceae).

Various Palmae are common with *Licuala kunstleri* Becc., *Pinanga maliana* (Mart.) Scheff., and *Salacca wallichiana* Mart., all treelets, and rattans, viz. *Calamus axillaris* Becc., *C. exilis* Griff., *C. javensis* Bl., *C. palustris* Griff. var. *cochinchinensis* Becc., *Daemonorops sabut* Becc., and *Korthalsia laciniosa* (Griff.) Mart.

Bamboos (Gramineae, Bambusoidese) are diverse and abundant in the swamp forest with *Gigantochloa apus* (Schult.) Kurz, *G. wrayi* Gamb., and *Cephalostachyum virgatum* (Munro) Kurz.

Many herbs are restricted to or are more common on tufa substrate, either epilithic or in thin soil. Some common examples are *Argostemma puffii* Srid. and *Ophiorrhiza trichocarpon* Bl. var. *trichocarpon* (both Rubiaceae), *Chirita involucrata* Craib and *Ephitema saxatile* Bl. (both Gesneriaceae)—all dicots. *Rhaphidophora gigantea* (Schott) Ridl. (Araceae) and *Nervilia aragoana* Gaud. (Orchidaceae) are two common monocot representatives. Some pteridophytes found in these places are *Nephrolepis biserrata* (Sw.) Schott (Oleandraceae), *Microsorum scolopendria* (Burm. f.) Copel. (Polypodiaceae), *Stenochlaena palustris* (Burm. f.) Bedd. (Pteridaceae, a vine), and *Schizaea digitata* (L.) Sw. (Schizaeaceae). The ground flora is also replete with seedlings and saplings of woody species.

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Treelets and shrubs are well-represented in the swamp forest, most of them being dicots. The most common species are *Ixora diversifolia* Wall. *ex* Kurz and *Saprosma longicalyx* Craib (both Rubiaceae), *Trevesia valida* Craib (Araliaceae), *Galeria fulva* (Tul.) Miq., *Phyllanthus albidiscus* (Ridl.) A.S., and *P. oxyphyllus* Miq.—the latter three Euphorbiaceae.

Understorey trees are common and include: *Garcinia merguensis* Wight (Guttiferae), *Sterculia guttata* Roxb. (Sterculiaceae), *Stemonurus malaccensis* (Mast.) Sleum. (Icacinaceae), *Saraca indica* L. (Leguminosae, Caesalpinioideae), *Carallia brachiata* (Lour.) Merr. (Rhizophoraceae), *Eugenia muelleri* Miq. and *E. oleina* Wight (Myrtaceae), *Madhuca malaccensis* (Cl.) Lam and *M. motleyana* (de Vr.) Baeh. (Sapotaceae), *Diospyros undulata* Wall. *ex* G. Don var. *cratericalyx* (Craib) Bakh. and *D. venosa* Wall. *ex* A. DC. var. *venosa* (Ebenaceae), and *Triadica cochinchinensis* Lour. (Euphorbiaceae).

Canopy trees, 30-40 m tall, are typically dense, often massive, and frequently have buttresses and pneumatophores. Some of the more common representatives are *Dipterocarpus kerrii* King (Dipterocarpaceae), *Canarium patentinervium* Miq. (Burseraceae), *Toona ciliata* M. Roem. (Meliaceae), *Pometia pinnata* J.R. & G. Forst. (Sapindaceae), *Parkia timoriana* (DC.) Merr. (Leguminosae, Mimosoideae), *Duabanga grandiflora* (Roxb. *ex* DC.) Walp. (Sonneratiaceae), *Eugenia operculata* Roxb. (Myrtaceae), *Horsfieldia brachiata* (King) Warb. (Myristicaceae), and *Ficus variegata* Bl. (Moraceae , Photo 5)

Several tree species found in the swamp forest are also found stunted, but reproductive, in open, wet tufa areas. The most common examples are *Eugenia papillosa* Duth. (Myrtaceae), *Alstonia macrophylla* Wall. *ex* G. Don (Apocynaceae), and *Raermachera pinnata* (Blanco) Steen. ssp. *acuminata* (Steen.) Steen. (Bignoniaceae). Other plants associated with tufa are *Arundinaria graminifolia* (D. Don) Hochr. (Orchidaceae) and *Lycopodium cernuum* L. (Lycopodiaceae)—herbs; *Psychotria sarmentosa* Val. (Rubiaceae) and *Nepenthes mirabilis* (Lour.) Druce (Nepentheaceae), both vines; and *Ligustrum confusum* Dcne. (Oleaceae), a treelet, shrub, or small tree.

Primary, evergreen, seasonal, hardwood + bamboo forest

This kind of forest facies is found above the swamp forest and mostly on sandstone bedrock above *c.* 25 m elevation. The flora of these two forest types is generally different mainly because of better drainage in the hardwood + bamboo areas.

The ground flora in this forest type is generally dense and evergreen. Herbs are plentiful with *Hedyotis pachycarp*a Ridl. (Rubiaceae), *Staurogyne merguensis* O.K. (Acanthaceae)—dicots; *Etlingera littoralis* (Kon.) Gise. and *Zingiber zerumbet* (L.) J.E. Sm. var. *zerumbet* (both Zingiberaceae)—monocots. Pteridophytes are represented with *Taenitis blechnoides* (Willd.) Sw. (Parkeriaceae), *Tectatia angulata* (Willd.) C. Chr. (Dryopteridaceae), and *Lygodium flexuosum* (L.) Sw. (Schizaeaceae, a vine). Seedlings and saplings of woody species are abundant.

Treelets and shrubs are numerous with: *Clausnea excavata* Burm. f. var. *excavata* (Rutaceae), *Leea indica* (Burm. f.) Merr. (Leeaceae), *Psychotria curviflora* Wall. (Rubiaceae), *Breynia vitis-ideae* (Burm. f.) C.E.C. Fisch. (Euphorbiaceae) —all dicots; and *Pandanus ovatus* (Gaud.) Kurz (Pandanaceae) —a monocot.

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Photo 5. *Ficus variegata* Bl. (Moraceae) , a canopy tree, along the Tina Jolliffe Nature Trail in the swamp forest. Buttresses and exposed, vertically flattened roots are common in this habitat.

Woody climbers are numerous and include: *Tectaria loureiri* (Fin. & Gagnep.) Pierre *ex* Craib (Dilleniaceae), *Uvaria cordata* (Dun.) Alst. (Annonaceae), *Entada rheedei* Spreng. (Leguminosae, Mimosoideae), *Aganope thyrsiflora* (Bth.) Polh. (Leguminosae, Papilionoideae), and *Urceola rosea* (Hk. & Arn.) Midd. (Apocynaceae).

The canopy of primary, evergreen, seasonal, hardwood + bamboo forest is as high as that in the swamp forest, but buttresses are less common and pneumatophores absent. Typical examples include: *Enicosanthum fuscum* (King) A.S. (Annonaceae), *Schima wallichii* (DC.) Korth. (Theaceae), *Irvingia malayana* Oliv. *ex* Benn. (Irvingiaceae), *Callerya atropurpurea* (Wall.) Schot (Leguminosae, Papilionoideae), *Tetrameles nudiflora* R. Br. *ex* Benn. (Datiscaceae, which is deciduous), *Cinnamomum iners* Reinw. *ex* Bl. and *Litsea grandis* (Wall. *ex* Nees) Hk. f. (both Lauraceae), *Chaetocarpus castanocarpus* (Roxb.) Thw. (Euphorbiaceae), *Castanopsis schefferiana* Hance and *Lithocarpus falconeri* (Kurz) Rehd. (both Fagaceae).

Bamboos (Gramineae, Bambusoideae) are well-represented with some species also found in the swamp forest. *Dinochloa scandens* (Bl.) O.K., a sprawling species, *Gigantochloa nigrociliata*

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(Buse) Kurz, and *Thyrsostachys oliveri* Gamb. are common in this forest type. *Orania sylvicola* (Griff.) H.E. Moore (Palmae), a conspicuous palm tree up to 20 m tall with pinnate leaves and a smooth trunk, is often seen on slopes.

Epiphytes

In addition to a profusion of fungi, algae, lichens, and bryophytes, vascular epiphytes are found in both kinds of forest. The amount of exposure and moisture, not soil or bedrock, determines the abundance and distribution of all epiphytes. Dicot representatives are sparse and include *Dischidia major* (Vahl) Merr. (Asclepiadaceae), *Macrosolen cochinchinensis* (Lour.) Tiegh. and *Scurrula parasitica* L. (both Loranthaceae and hemi-parasitic shrubs). Monocots include several Orchidaceae, e.g. *Cymbidium* sp. (flowers not seen) and *Dendrobium secundum* (Bl.) Lindl. Pteridophytes are most frequently seen with *Davallia divaricata* Bl. (Davalliaceae), *Asplenium nidus* L. var. *nidus* (Aspleniaceae), and Polypodiaceae with *Aglaomorpha coronans* (Wall. *ex* Mett.) Copel., *Drynaria quercifolia* (L.) J. Sm., and *Microsorum punctatum* (L.) Copel.

Disturbed areas and secondary growth

Primary succession in cleared areas or large gaps in the forest include an initial invasion of herbaceous weeds—all of which are common throughout South-East Asia. Some of the most common species include: *Ageratum conyzoides* L. *and Eupatorium odoratum* L. (both naturalised Compositae), *Phyllanthus urinaria* L. and *P. amarus* Schum. & Thon. (Euphorbiaceae) ―dicots. Monocots are very diverse and abundant with many Cyperaceae, e.g. *Cyperis kyllingia* Endl., *C. iria* L., and *Fimbristylis dichotoma* (L.) Vahl ssp. *dichotoma*; and Gramineae, viz. *Imperata cylindrica* (L.) P. Beauv. var. *major* (Nees) C.E. Hubb. *ex* Hubb. & Vaugh., *Eragrostis pilosa* (L.) P. Beauv., *Cyrtococcum oxyphyllum* (Steud.) Stapf, *Panicum maximum* Jacq., and *Phragmites vallatoria* (Pluk. *ex* L.) Veldk. *Musa acuminata* Colla ssp. *siamea* Simm. (Musaceae), a wild banana, and *Dicranopteris linearis* (Burm. f.) Underw. var. *linearis* (Gleicheniaceae, a pteridophyte) are also common.

Woody species often develop with the herbaceous weeds and eventually replace them by shading. *Ziziphus oenoplia* (L.) Mill. (Rhamnaceae, a spiny woody climber) and *Melastoma malabathricum* L. ssp. *malabathricum* (Melastomataceae, a treelet) as well as trees are common, viz. *Eurya acuminata* DC. var. *acuminata* (Theaceae), *Microcos paniculata* L. (Tiliaceae), *Callicarpa arborea* Roxb. var. *arborea* and *Vitex quinata* (Lour.) Will. (both Verbenaceae), *Macaranga denticulata* (Bl.) M.A. (Euphorbiaceae), *Trema orientalis* (L.) Bl. (Ulmaceae), and *Ficus hispida* L.f. (Moraceae).

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APPENDIX
Plant Database

1) **Summary of collecting**

Division	Subdivision	Class	Families	Species, *etc.*
Spermatophyta (seed plants)	Angiospermae	Dicotyledoneae	77	295
		Monocotyledoneae	19	90
	Gymnospermea e		1	1
Pteridophyta (fern allies & ferns)			14	34
		Total	**111**	**420**

2) **List of abbreviations used in the database**

LIFE MODE: gro ground; int introduced/not native; nat naturalised; hemipar hemiparasite; sap saprophyte; str "strangler"; wee weed; cul cultivated; epi epiphyte; epl epilithic

HABIT: t tree; l treelet; wc woody climber; s shrub; v vine; h herb; sc scandent; cr creeping

APED: a annual; pe perennial evergreen; pd perennial deciduous

ABUNDANCE:
0 Probably extirpated
1 Down to a few individuals, in danger of extirpation
2 Rare
3 Medium abundance
4 Common, but not dominant
5 Abundant

HABITAT:
evergreen forest egf
evergreen forest with bamboo eg/bb
disturbed areas, roadsides da
secondary growth sg

BEDROCK: gr granite; is limestone; ss sandstone

FLOWERING FRUITING AND LEAFING MONTHS: ja fb mr ap my jn jl ag sp oc nv de (January – December)

3) **Database proper (p. 12-25)**

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SPECIES	FAMILY	LIFE MODE	HABIT	APED	ABUN-DANCE	HABITAT	BED-ROCK	LOW ALT(M)	UPPER ALT(M)	FLOWER_ MTH	FRUIT_ MTH	LEAF_ MTH	FLOWER	FRUIT	Coll_NO.
ANGIOSPERMAE, DICOTYLEDONEAE															
Acrotrema costatum Jack	Dilleniaceae	gro	h	pe	3	eg/bb	ls	50	100	ag-sp		ja-dc	y		07-610
Dillenia obovata (Bl.) Hoogl.	Dilleniaceae	gro	t	pe	3	eg/bb	ls	25	75	mr-ap		ja-dc	y		06-185
Tetracera loureiri (Fin. & Gagnep.) Pierre *ex* Craib	Dilleniaceae	gro	wc	pe	3	eg/bb	ls ss	25	125			ja-dc			
Magnolia chanpaca (L.) Baill. *ex* Pierre var. *chanpaca*	Magnoliaceae	gro	t	pe	2	egf	ss	30	175	ja-fb		ja-dc			
Artabotrys suaveolens (Bl.) Bl.	Annonaceae	gro	wc	pe	3	eg/bb	ls	50	100	ja-fb		ja-dc	y		06-42
Desmos dasymachalus (Bl.) Saff. var *dasymaschalus*	Annonaceae	gro	l	pe	2	egf eg/bb	ls	25	75		ag	ja-dc			
Enicosanthum fuscum (King) A.S.	Annonaceae	gro	t	pe	3	eg/bb	ss	25	50	mr-ap		ja-dc	y		06-241
Mezzettia curtisii King	Annonaceae	gro	t	pe	2	eg/bb	ss	25	75		ag-sp	ja-dc			07-612
Miliusa amplexicaulis Ridl.	Annonaceae	gro	l	pe	2	rocks, cliffs eg/bb	ls	75	100	sp-oc	sp-oc	ja-dc			05-551
Orophea malayana Kess.	Annonaceae	gro	t,l	pe	3	eg/bb	ls	25	75	mr-ap		ja-dc	y		06-244
Polyalthia jenkensii (Hk.f.&Th.) Hk.f.&Th.	Annonaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Uvaria cordata (Dun.) Alst.	Annonaceae	gro	wc	pe	3	eg/bb da sg	ls ss	25	150	ag-sp		ja-dc			06-570
Archangelisia flava (L.) Merr.	Menispermaceae	gro	wc	pe	3	streams in eg/bb	ls	25	75	sp-oc		ja-dc	male		05-495
Pseuduvaria macrophylla (Oliv.) Merr. var. *macrophylla*	Annonaceae	gro	l	pe	3	streams in eg/bb	ls	50	75	ja-fb		ja-dc	y		06-22
Pseuduvaria rugosa (Bl.) Merr.	Annonaceae	gro	t	pe	3	eg/bb	ls	100	125	ja-fb		ja-dc	y		06-50
Epirixanthes elongata Bl.	Polygalaceae	gro sap	h	a	2	wet areas in eg/bb	ls	25	75	mr-ap		mr-jl			
Rinorea sclerocarpa (Berq.) Jacobs	Violaceae	gro	l	pe	2	streams in eg/bb	ls	25	50	sp-mr	oc-ap	ja-dc			05-498
Salomonia cantoniensis Lour.	Polygalaceae	gro	h	a	3	da	ls	25	50	ag-oc	sp-nv	my-nv			05-500
Calophyllum soulattri Burm.f.	Guttiferae	gro	t	pe	3	eg/bb	ls	50	100		ja-ap	ja-dc		y	06-19
Calophyllum tetrapterum Miq.	Guttiferae	gro	t	pe	3	eg/bb	ls ss	50	100	dc-ja	mr-ap	ja-dc	y	y	06-55, 06-201
Cratoxylum cochinchinense (Lour.) Bl.	Guttiferae, Hypericaceae	gro	t	pe	3	da sg	ss	25	100		ag-sp	ja-dc			06-590
Cratoxylum formosum (Jack) Dyer															
ssp. *pruniflorum* (Kurz) Gog.	Guttiferae, Hypericaceae	gro	t(l)	pd	3	eg/bb da sg	ls ss	25	100	mr-ap		ap-mr	y		06-240
Garcinia hombroniana Pierre	Guttiferae	gro	t	pe	3	eg/bb	ls	25	75		mr-ap	ja-dc		x	06-205
Garcinia merguensis Wight	Guttiferae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75	mr-ap	sp-oc	ja-dc	male	imm	05-542,06-177
Garcinia rostrata (Hassk.) Miq.	Guttiferae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Mesua ferrea L.	Guttiferae	gro	t	pe	2	egf eg/bb	ss	25	75		jn-jl	ja-dc			s-75
Flacourtia indica (Burm.f.) Merr.	Flacourtiaceae	gro	t(l,s)	pd	3	wet areas in eg/bb	ls	25	75			my-fb			
Homalium dasyanthum (Turcz.) Warb.	Flacourtiaceae	gro	t	pe	3	eg/bb da	ls	25	75	mr-ap		ja-dc	y		06-186
Scolopia spinosa (Roxb.) Warb.	Flacourtiaceae	gro	t	pe	3	eg/bb	ls	50	100	dc-ja	ap-my	ja-dc	y	y	06-40,06-219

Eurya acuminata DC. var. *acuminata*	Theaceae	gro	t	pe	3	da sg	ss	25	225		ag-sp	ja-dc			06-577
Eurya nitida Korth. var *siamensis* (Craib) H.Keng	Theaceae	gro	t(l)	pe	3	eg/bb	ls	50	100		ja-fb	ja-dc		y	06-44
Schima wallichii (DC.) Korth.	Theaceae	gro	t	pe	3	eg/bb	ls	25	75	mr		ja-dc			
Anisoptera scaphula (Roxb.) Pierre	Dipterocarpaceae	gro	t	pe	2	egf eg/bb	ss	25	175		jn-jl	ja-dc			s-78
Cotylelobium melanoxylon Pierre	Dipterocarpaceae	gro	t	pe	2	eg/bb	ls	50	125		ap	ja-dc		y	06-221
Dipterocarpus dyeri Pierre *ex* Lanes.	Dipterocarpaceae	gro	t	pe	3	eg/bb	ls	25	75		mr-ap	ja-dc			
Dipterocarpus kerrii King	Dipterocarpaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Hopea odorata Roxb. var. *odorata*	Dipterocarpaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Shorea foxworthii Sym.	Dipterocarpaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Vatica odorata (Griff.) Sym.	Dipterocarpaceae	gro	t	pe	3	eg/bb	ls	25	75			ja-dc			
Vatica stapfiana (King) Sloot.	Dipterocarpaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75	sp-oc	mr	ja-dc	y		05-546
Hibiscus macrophyllus Roxb. *ex* Horn.	Malvaceae	gro	t	pe	3	da sg	ls ss	75	150			ja-dc			
Durio griffithii (Mast.) Bakh.	Bombacaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Leptonychia caudata (Wall. *ex* D. Don) Burr.	Sterculiaceae	gro	l	pe	2	eg/bb	ls	100	125	ja-fb		ja-dc	y		06-51
Pterocymbium tinctorium (Blanco) Merr.	Sterculiaceae	gro	t	pd	3	wet areas in eg/bb	ls	25	75		mr-ap	ap-fb			
Pterospermum sp.	Sterculiaceae	gro	t	pe	3	egf eg/bb	ls	25	75			ja-dc			tree 52
Sterculia guttata Roxb.	Sterculiaceae	gro	t	pe	3	streams, wet areas in eg/bb, swamp forest	ls	25	75			ja-dc			tree 81
Grewia acuminata Juse.	Tiliaceae	gro	wc	pe	3	da	ls	25	175		ag-sp	ja-dc			07-629
Microcos paniculata L.	Tiliaceae	gro	t(l)	pd	3	wet areas in eg/bb da sg	ls ss	25	75		ag-oc	ja-dc			
Elaeocarpus petiolatus (Jack) Wall. *ex* Kurz	Elaeocarpaceae	gro	t	pe	3	eg/bb	ls	50	125	ag	dc-ja	ja-dc		y	06-74
Elaeocarpus stipularis Bl.	Elaeocarpaceae	gro	t	pd	3	eg/bb	ls	25	75		oc-nv	ja-dc		imm.	05-545
Erythroxylum cuneatum (Miq.) Kurz	Erythroxylaceae	gro	s(t)	pe	2	eg/bb	ls ss	50	125		ja-fb	ja-dc			forru 290
Atalantia monophylla (L.) DC.	Rutaceae	gro	t(l)	pe	3	eg/bb da	ls	25	50		mr-ap	ja-dc		x	06-183
Clausena excavata Burm.f. var. *excavata*	Rutaceae	gro	l(t)	pe (pd)	3	eg/bb da sg	ls ss	25	75	mr-ap		ja-dc	x		06-235
Glycosmis pentaphylla (Retz.) DC. var. *pentaphylla*	Rutaceae	gro	l	pe	3	egf eg/bb	ss	25	175			ja-dc			
Luvunga scandens (Roxb.) Ham. *ex* Wight	Rutaceae	gro	wc	pe	3	da sg	ss	25	150	ag-sp	ag-sp	ja-dc			06-557
Micromelum falcatum (Lour.) Tana.	Rutaceae	gro	l(s)	pe	3	eg/bb	ls	50	100	dc-fb		ja-dc	y		06-53
Murraya paniculata (L.) Jack	Rutaceae	gro	l	pd	3	rocks in egf eg/bb	ls ss	25	175			ja-dc			
Tetradium glabrifolium (Champ. *ex* Bth.) T. Hart.	Rutaceae	gro	t	pe	2	da sg	ss	75	125	ag-sp		ja-dc			06-588
Zanthoxylum rhetsa (Roxb.) DC.	Rutaceae	gro	t	pd	2	eg/bb da sg	ls ss	25	100	mr-ap		ap-fb	male		06-220
Eurycoma longifolia Jack	Simaroubaceae	gro	l	pd	2	eg/bb	ls ss	50	125			ja-dc			
Irvingia malayana Oliv. *ex* Benn.	Irvingiaceae	gro	t	pe	3	eg/bb sg	ss	50	150			ja-dc			
Canarium patentinervium Miq.	Burseraceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75	mr	ap-my	ja-dc			
Gomphandra quadrifida (Bl.) Sleum. var.	Icacinaceae	gro	l(s)	pe	3	streams in eg/bb	ls	25	75		sp-oc	ja-dc		y	05-496

quadrifida															
Lansium domesticum Corr.	Meliaceae	gro	t	pe	2	eg/bb	ls	75	100	my-jn		ja-dc			tree 95
Sandoricum koetjape (Burm. f.) Merr.	Meliaceae	gro	t	pe	3	egf eg/bb	ss	25	175			ja-dc			s-51
Toona ciliata M.Roem.	Meliaceae	gro	t	pe	3	eg/bb	ls	75	100	mr	jl-ag	ja-dc			
Stemonurus malaccensis (Mast.) Sleum.	Icacinaceae	gro	t	pe	3	wet areas in eg/bb, swamp forest	ls	25	75	ag-sp		ja-dc			06-571
Ilex sp.	Aquifoliaceae	gro	t	pd	3	eg/bb	ls	25	75	my-jn		my-mr	imm.		06-202
Bhesa paniculata Arn.	Celastraceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Gouania javanica Miq.	Rhamnaceae	gro	wc	pe	3	streams in eg/bb sa	ls	50	100	dc-ja		ja-dc	y		06-2
Ziziphus oenoplia (L.) Mill.	Rhamnaceae	gro	wc	pe	3	da sg	ls ss	25	225			ja-dc			
Cayratia japonica (Thunb.) Gagnep.	Vitaceae	gro	v	a	3	egf eg/bb da sg	ls ss	25	125	ag-sp		ja-dc	y		06-542
Cissus convolvulacea Planch.	Vitaceae	gro	v (wc)	pd	3	eg/bb da sg	ls	50	100		ja-fb	ja-dc		y	06-43
Leea indica (Burm. f.) Merr.	Leeaceae	gro	t(l)	pe	3	eg/bb da sg	ls ss	25	150		jl-sp	ja-dc			06-573
Allophyllus cobbe (L.) Raeus.	Sapindaceae	gro	l	pe	2	eg/bb, swamp forest	ls	25	75	ag-sp		ja-dc			
Harpullia cupanioides Roxb.	Sapindaceae	gro	t	pe (pd)	3	wet areas in eg/bb	ls	25	75	mr-ap		ja-dc	male		06-213
Lepisanthes rubiginosa (Roxb.) Leenh.	Sapindaceae	gro	t(l)	pe(pd)	3	eg/bb	ls	25	50	mr-ap		ja-dc	male		06-179
Nephelium hypoleucum Kurz	Sapindaceae	gro	t	pe	3	eg/bb	ss	25	175			ja-dc			s-50
Pometia pinnata J.R. Forst. & G. Forst.	Sapindaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75	mr-ap	ag-oc	ja-dc		y	07-618
Buchanania arborescens (Bl.) Bl.	Anacardiaceae	gro	t	pe	3	eg/bb	ls	50	100	ja-fb	mr-ap	ja-dc	y	y	06-56, 06-232
Mangifera linearifolia Kosterm.	Anacardiaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Semecarpus cochinchinensis Engl.	Anacardiaceae	gro	t	pe	3	eg/bb	ls	50	100	ja-fb		ja-dc	male		06-20
Archidendron contortum (Mart.) l. Niels.	Leguminosae, Mimosoideae	gro	t	pe	3	eg/bb	ls	25	75		mr-ap	ja-dc			
Entada spiralis Ridl.	Leguminosae, Mimosoideae	gro	wc	pd	3	streams in eg/bb	ls	25	50	mr-ap		ja-dc	y		06-175
Parkia timoriana (DC.) Merr.	Leguminosae, Mimosoideae	gro	t	pe	3	eg/bb, swamp forest	ls	25	75			ja-dc			s-29
Bauhinia bassacensis Pierre *ex* Gagnep. var. *bassacensis*	Leguminosae, Caesalpinioideae	gro	wc	pe	3	streams in eg/bb	ls	50	75	ja-fb		ja-dc	y		06-57
Dialium indum L.	Leguminosae, Caesalpinioideae	gro	t	pe	3	egf eg/bb	ss	25	175		jn-jl	ja-dc			s-72
Peltophorum pterocarpum (DC.) Back.*ex* K.Hey.	Leguminosae, Caesalpinioideae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Saraca indica L.	Leguminosae, Caesalpinioideae	gro	t(s)	pe	4	streams, ponds,wet areas in eg/bb	ls	25	75	sp-fb		ja-dc	y		05-549
Aganope thyrsiflora (Bth.) Polh.	Leguminosae, Papilionoideae	gro	wc	pe	3	streams in eg/bb	ls ss	50	75	ja-fb		ja-dc	y		06-67
Callerya atropurpurea (Wall.) Schot	Leguminosae, Papilionoideae	gro	t	pe	3	eg/bb	ls ss	25	150	mr-ap	jl-sp	ja-dc	y	y	06-170, 06-581
Calopogonium mucunoides Desv.	Leguminosae, Papilionoideae	gro wee	v	a (pe)	3	da sg	ls ss	50	100	nv-fb	fb-mr	ja-dc	y		06-64
Desmodium heterocarpon (L.) DC. ssp. *heterocarpon*															

var. *strigosum* Mee.	Leguminosae, Papilionoideae	gro	h	pe	3	da sg	ls	50	100	dc-ja		ja-dc	y		06-14
Flemingia stricta Roxb. *ex* Ait.f.	Leguminosae, Papilionoideae	gro	s	pe	3	da sg	ls	50	100	dc-ja	ja-fb	ja-dc	y	y	06-3
Pueraria phaseoloides (Roxb.) Bth. var. *phaseoloides*	Leguminosae, Papilionoideae	gro	v	a(pe)	3	da sg	ls	50	75	dc-mr	fb-ap	ja-dc	y		06-4
Eriobotrya bengalensis (Roxb.) Hk.f. forma *bengalensis*	Rosaceae	gro	t	pe	3	eg/bb	ls	50	100	dc-ja		ja-dc	y		06-72
Maranthes corymbosa Bl.	Chrysobalanaceae (Rosaceae)	gro	t	pe	3	streams in eg/bb	ls	25	50	mr-ap		ja-dc	y		06-176
Carallia brachiata (Lour.) Merr.	Rhizophoraceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75			ja-dc			
Calycopteris floribunda (Roxb.) Lmk.	Combretaceae	gro	wc (sc)	pd	3	eg/bb da sg	ls	25	50	fb-mr	mr-ap	my-mr		y	06-196
Combretum latifolium Bl.	Combretaceae	gro	wc	ped	2	streams, wet areas in eg/bb	ls	50	75	ja-fb		ja-dc			
Terminalia citrina (Gaertn.) Roxb. *ex* Flem.	Combretaceae	gro	t	pe	3	eg/bb	ls	25	75	mr-ap		ja-dc	y		06-178
Eugenia borneensis Miq. var. *borneensis*	Myrtaceae	gro	t	pe	3	eg/bb	ls	25	75	ap-my		ja-dc	y		06-224
Eugenia cerasiformis (Bl.) DC.	Myrtaceae	gro	t	pe	3	eg/bb	ls	25	75			ja-dc			
Eugenia claviflora Roxb. var. *claviflora*	Myrtaceae	gro	t	pe	3	eg/bb	ls	50	100	ja-fb		ja-dc	y		06-73
Eugenia cumini (L.) Druce	Myrtaceae	gro	t	pe(pd)	2	eg/bb	ls	25	75		mr-ap	ja-dc		y	06-215
Eugenia grandis Wight var. *grandis*	Myrtaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75	fb		ja-dc			
Eugenia grata Wight var. *grata*	Myrtaceae	gro	t	pe	2	eg/bb egf	ls ss	25	225			ja-dc			
Eugenia muelleri Miq.	Myrtaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	50	75	ag		ja-dc			06-529
Eugenia oleina Wight	Myrtaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75	sp-oc	mr-ap	ja-dc	y		05-533
Eugenia operculata Roxb.	Myrtaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	125			ja-dc			
Eugenia papillosa Duth.	Myrtaceae	gro	t	pe	4	streams, wet areas in eg/bb	ls	25	100	ja-fb		ja-dc	y		06-49
Eugenia syzygioides (Miq.) Hend.	Myrtaceae	gro	t	pe	3	eg/bb	ls	50	100	ja-fb		ja-dc	y		06-59
Rhodamnia cinerea Jack var. *cinerea*	Myrtaceae	gro	t	pe	3	eg/bb	ss	75	150	jl-ag	ag-sp	ja-dc			06-585
Rhodomyrtus tomentosa (Ait.) Hassk.	Myrtaceae	gro	l	pe	3	da sg	ls	50	75	dc-fb	fb-mr	ja-dc	y		06-61
Melastoma malabathricum L. ssp. *malabathricum*	Melastomataceae	gro	l	pe	3	da sg	ls ss	50	100	ja-dc	ja-dc	ja-dc			
Memecylon corticosum Ridl.	Melastomataceae	gro	l	pe	2	rocks in eg/bb	ls	25	175	jl-sp	sp-oc	ja-dc			07-632
Memecylon sp.	Melastomataceae	gro	l	pe	2	eg/bb, swamp forest	ls	25	75			ja-dc			
Lagerstroemia floribunda Jack var. *floribunda*	Lythraceae	gro	t	pd	3	da sg	ls	25	75	ag-oc		ja-dc	y		05-514
Crypteronia paniculata Bl. var. *paniculata*	Crypteroniaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Duabanga grandiflora (Roxb. *ex* DC.) Walp.	Sonneratiaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75	mr		ja-dc			
Adenia penangiana (Wall. ex G. Don) Wilde															
var. *parvifolia* (Pierre ex Gagnep.) Wilde	Passifloraceae	gro	v	a(pe)	2	streams, wet areas in eg/bb	ls	50	75	mr-ap		mr-dc	male		06-216
Momordica cochinchinensis (Lour.) Spreng.	Cucurbitaceae	gro	wc	pe	2	egf ,eg/bb	ls	50	100	ag		ja-dc			
Begonia aff. *brandisiana* Kurz	Begoniaceae	epl	h	pd	3	rocks in eg/bb	ls	25	175	ag-sp	sp-oc	my-dc			07-631

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Begonia curtisii Ridl.	Begoniaceae	epl	t(h)	pd	2	rocks, cliffs in eg/bb	ls	25	75	ag-oc	oc-nv	my-dc	y	y	05-541
Tetrameles nudiflora R.Br. *ex* Benn.	Datiscaceae	gro	t	pd	3	eg/bb	ls	75	100			my-fb			tree 91
Schefflera sp.	Araliaceae	epi	s	pe	3	streams, wet areas in eg/bb, swamp forest	ls	50	75			ja-dc			
Trevesia valida Craib	Araliaceae	gro	t(l)	pe	3	streams, wet areas in eg/bb	ls	25	75		sp-oc	ja-dc		y	05-553
Anthocephalus chinensis (Lmk.) A. Rich. *ex* Walp.	Rubiaceae	gro	t	pe	3	da sg	ls	25	75		ag-sp	ja-dc		y	06-561
Argostemma puffii Srid.	Rubiaceae	epl	h	pd	3	rocks, cliffs in eg/bb	ls	25	100		oc-nv	my-dc		y	05-525
Canthium glabrum Bl.	Rubiaceae	gro	t	pe	3	eg/bb	ls	25	75			ja-dc			
Canthium sp.	Rubiaceae	gro	l	pe	3	egf, eg/bb	ls	25	125			ja-dc			
Fagerlindia fasciculata (Roxb.) Tirv.															
var. *parviflora* (Gamb.) Wong	Rubiaceae	gro	l	pe	2	eg/bb da	ls	25	50	mr-ap	sp-oc	ja-dc	y	y	06-192, 07-634
Gardenia griffithii Hk. f.	Rubiaceae	gro	t	pe	2	eg/bb	ss	75	175		ag-sp	ja-dc			07-635
Hedyotis pachycarpa Ridl.	Rubiaceae	gro wee	h	a	3	da sg	ss	50	125	jl-sp	ag-oc	my-dc			06-555
Hedyotis philippensis (Willd.) Merr. *ex* C.B. Rob.	Rubiaceae	gro	l	pe	2	eg/bb da sg	ls ss	50	100	ag-sp	ja-fb	ja-dc	y	y	06-54,06-547
Hedyotis wallichii Kurz	Rubiaceae	gro	h	a	3	da	ls	25	75	sp-oc		my-dc	y		05-538
Hypobathrium racemosum (Roxb.) Kurz	Rubiaceae	gro	t(l)	pd	3	streams in eg/bb	ls	25	75	sp-oc	oc-nv	ja-dc	y	y	05-497, 05-520
Ixora diversifolia Wall. *ex* Kurz	Rubiaceae	gro	l	pe	3	streams, wet areas in eg/bb	ls	25	75	ap	sp-oc	ja-dc		y	05-540
Ixora javanica (Bl.) DC.	Rubiaceae	gro	l	pe	3	eg/bb	ls	25	75	mr-ap(ag)		ja-dc	y		06-169
Ixora multibracteata Pear. *ex* King & Gamb.	Rubiaceae	gro	t,l	pe	3	streams in eg/bb	ls	25	75	mr-ap	sp	ja-dc	y		06-174
Lasianthus kurzii Hk. f. var. *kurzii*	Rubiaceae	gro	l	pe	2	eg/bb	ss	75	125		ag	ja-dc			06-586
Morinda elliptica (Hk.f.) Ridl.	Rubiaceae	gro	t	pe	3	eg/bb	ls	25	75	mr-oc	oc-nv	ja-dc	y		05-554
Mussaenda polyneura King	Rubiaceae	gro	wc	pe	3	eg/bb da sg	ls	25	75	mr-ap	sp-oc	ja-dc		y	05-534
Mycetia malayana (G. Don) Craib	Rubiaceae	gro	s	pe	2	eg/bb	ls	50	75	ag	sp-oc	ja-dc		y	05-512
Ophiorrhiza trichocarpon Bl. var. *trichocarpon*	Rubiaceae	gro	h	pd	3	wet areas in eg/bb	ls	25	75	ag-mr	oc-ap	ja-dc	y		05-504
Paederia scandens (Lour.) Merr.	Rubiaceae	gro	v	pe	3	eg/bb da	ls ss	50	100		dc-ja	ja-dc		y	06-13
Psychotria angulata Korth.	Rubiaceae	gro	l	pe	3	eg/bb	ls	50	100		ja-fb	ja-dc		y	06-41
Psychotria curviflora Wall.	Rubiaceae	gro	l	pe	3	eg/bb	ls ss	25	125	mr-ap	jl-sp	ja-dc	y	y	06-203,06-572
Psychotria rhinocerotis Reinw. *ex* Bl.	Rubiaceae	gro	l	pe	3	wet areas in eg/bb	ls	25	75	mr-ap	ag	ja-dc	x		06-212
Psychotria sarmensoides Val.	Rubiaceae	gro	v(cr)	pe	3	eg/bb	ls	50	100	mr-ap	dc-ja(ag)	ja-dc	y	y	06-1,06-207
Psychotria stipulacea Wall. var. *stipulacea*	Rubiaceae	gro	l	pe	2	eg/bb	ls ss	50	100	ja-fb	ag	ja-dc	y	y	06-36,06-554
Rennellia speciosa Hk. f.	Rubiaceae	gro	l	pe	2	wet areas in eg/bb	ls ss	25	125		ag-sp	ja-dc			06-545
Rothmannia schoemannii (Teijsm. & Binn.) Tirv.	Rubiaceae	gro	t	pe	3	eg/bb	ss	75	150		ag-sp	ja-dc			06-587
Saprosma longicalyx Craib	Rubiaceae	gro	l	pe	3	wet areas in eg/bb, egf, swamp forest	ls	25	75		ag-sp	ja-dc			06-540
Tarenna sp.	Rubiaceae	gro	l	pe	3	egf, eg/bb, swamp forest	ls	25	75		ag-sp	ja-dc			06-541

Timonius wallichianus (Korth.) Val.	Rubiaceae	gro	t	pe	2	eg/bb	ls	50	100		ja-fb	ja-dc		y	06-46
Elephantopus scaber L. ssp. *scaber* var. *scaber*	Compositae	gro	h	pe	3	da sg	ls	50	75	dc-fb	fb-mr	ja-dc	y		06-48
Eupatorium odoratum L.	Compositae	gro nat wee	h	a(pe)	3	eg/bb da sg	ls	50	100	ja-fb		ja-dc			
Mikania cordata (Burm.f.) B.L. Rob. forma undulata Kost.	Compositae	nat (gro wee)	v	a	3	da sg	ls	50	75	ja-fb		ja-dc			
Ardisia crenata Sims var. *crenata*	Myrsinaceae	gro	l	pe	3	da sg	ls	50	125	dc-ja	ag	ja-dc			
Ardisia quinquegona Bl.	Myrsinaceae	gro	l	pe	3	streams, wet areas in eg/bb	ls	50	75		ja-fb	ja-dc		y	06-24
Ardisia sanguinolenta Bl. var . *sanguinolenta*	Myrsinaceae	gro	t	pe	3	eg/bb	ls	25	75		mr-ap	ja-dc		y	06-246
Maesa ramentacea (Roxb.) A.DC.	Myrsinaceae	gro	t(l)	pe	3	da sg	ls	50	100	ja-fb		ja-dc	y		06-80
Madhuca malaccensis (Cl.) Lam	Sapotaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	50	100	ja-fb	ag	ja-dc	y	y	06-34, 06-528
Madhuca motleyana (de Vr.) Baeh.	Sapotaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Mimusops elengi L.	Sapotaceae	gro	t	pe	2	eg/bb	ls	50	75	jn	ag	ja-dc			
Pouteria obovata (R.Br.) Baeh.	Sapotaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75	mr-ap		ja-dc	y		06-217
Diospyros malabarica (Desr.) Kostel.															
var. *siamensis* (Hochr.) Pheng.	Ebenaceae	gro	t	pe	3	eg/bb	ls	25	75		ja-fb	ja-dc			
Diospyros undulata Wall. *ex* G. Don															
var. *cratericalyx* (Craib) Bakh.	Ebenaceae	gro	t	pe	3	streams, wet areas in eg/bb, egf	ls ss	25	125		sp-nv	ja-dc		y	05-539
Diospyros venosa Wall.*ex* A. DC. var. *venosa*	Ebenaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75	oc-nv		ja-dc	male		05-544
Symplocos celastrifolia Griff. *ex* Cl.	Symplocaceae	gro	t	pe	3	eg/bb	ls	25	75	mr-ap		ja-dc	y		06-204
Symplocos cochinchinensis (Lour.) S.Moore															
ssp. *cochinchinensis* var.*cochinchinensis*	Symplocaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Symplocos cochinchinensis (Lour.) S.Moore															
ssp. *laurina* (Retz.) Noot.	Symplocaceae	gro	t	pe	3	eg/bb	ls ss	25	175		sp-oc	ja-dc			07-617
Symplocos sumunita B.H. ex D. Don	Symplocaceae	gro	t(l)	pe	3	eg/bb	ls	25	75	ap-my		ja-dc	y		06-223
Styrax betongensis Flet.	Styracaceae	gro	t	pe	2	eg/bb	ls	25	175		jl-ag	ja-dc			07-630
Chionanthus calophyllus Bl.	Oleaceae	gro	t	pe	2	egf eg/bb	ss	25	175			ja-dc			s-30
Chionanthus ramiflorus Roxb.	Oleaceae	gro	t	pe	3	eg/bb	ls	25	100			ja-dc			
Jasminum nervosum Lour.	Oleaceae	gro	v	pe	3	eg/bb	ls	25	75	mr-ap		ja-dc	y		06-234
Jasminum rambayense O.K.	Oleaceae	gro	wc	pe	3	ponds in eg/bb sg	ls	25	50	mr-ap		ja-dc			06-194
Ligustrum confusum Dcne.	Oleaceae	gro	t(l,s)	pe	3	streams, ponds,wet areas in eg/bb	ls	50	75	ja (sp)	oc-nv	ja-dc	y	imm	05-518
Aganosma marginata (Roxb.) G.Don	Apocynaceae	gro	wc	pe(pd)	3	eg/bb da sg	ls	25	75	mr-ap		ja-dc	y		06-228
Alstonia macrophylla Wall.*ex* G.Don	Apocynaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75		ja-fb	ja-dc		y	06-75
Alstonia scholaris (L.) R. Br.	Apocynaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Alyxia reinwardtii Bl.	Apocynaceae	gro	wc	pe	3	eg/bb da sg	ls	25	75	ja-fb		ja-dc		y	06-200

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Kopsia fruticosa (Ker) A. DC.	Apocynaceae	gro	sh	pe	2	egf	ss	30	75	ja-fb					
Urceola rosea (Hk. & Arn.) Middl.	Apocynaceae	gro	wc	pe	3	eg/bb da sg	ls ss	25	150	mr-ap	ag	ja-dc	y	y	06-188, 06-544
Willughbeia edulis Roxb.	Apocynaceae	gro	wc	pe	3	eg/bb da sg	ls	25	75	mr-ap	mr-ap	ja-dc	y	y	06-227
Dischidia major (Vahl) Merr.	Asclepiadaceae	epi	cr	pe	3	streams, wet areas in eg/bb	ls	25	75	mr-ap		ja-dc			
Marsdenia tinctoria R. Br.	Asclepiadaceae	gro	v	pe	3	da	ls	25	175	ag-sp		ja-dc			07-628
Streptocaulon juventas (Lour.) Merr.	Asclepiadaceae	gro	v	pe	3	da sg	ls ss	25	150			ja-dc			
Tylophora tenuis Bl.	Asclepiadaceae	gro	v	pe(a)	3	eg/bb da sg	ls	25	75	mr-ap		ja-dc	y		06-195
Fagraea fragrans Roxb.	Loganiaceae	gro	t	pe	3	eg/bb da	ls	25	50	mr-ap	sp-oc	ja-dc	y	y	06-242, 07-608
Canscora pentanthera Cl.	Gentianaceae	gro	h	pe	3	streams, wet areas in eg/bb	ls	25	75	sp-ja	ja-fb	ja-dc	y		05-516
Argyreia capitiformis (Poir.) Oost.	Convolvulaceae	gro	v(wc)	pe	3	da sg	ls	50	75	dc-ja		ja-dc	y		06-17
Solanum macrodon Wall. *ex* Nees	Solanaceae	gro	l	pe	2	eg/bb da	ls	50	100	jn-ag	ag-sp	ja-dc			06-534
Adenosma indiana (Lour.) Merr.	Scrophulariaceae	gro	h	a	3	wet areas in eg/bb da sg	ls	50	100	dc-ja	ja-fb	jn-dc	y		06-6
Chirita involucrata Craib	Gesneriaceae	gro	h	a	3	streams, ponds,wet areas in eg/bb	ls	25	75	sp-fb	nv-fb	my-mr	y		05-508
Epithema saxatile Bl.	Gesneriaceae	epl	h	a	3	rocks, cliffs eg/bb	ls	50	100	ag-ja	oc-fb	my-fb	y		05-511
Oroxylum indicum (L.) Kurz	Bignoniaceae	gro	t(l)	pd	3	eg/bb da, wet areas	ls	25	75	mr		ja-dc			
Pajanelia longifolia (Willd.) K. Sch.	Bignoniaceae	gro	t	pd	3	wet areas in eg/bb	ls	25	75			ja-dc			
Radermachera pinnata (Blanco) Steen. ssp. *acuminata* (Steen.) Steen.	Bignoniaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75	mr	sp-oc	ja-dc		y	05-531
Andrographis laxiflora (Bl.) Lindau	Acanthaceae	gro	h	a (pe)	3	rocks, streams in eg/bb	ls	50	125	ja-fb	fb-mr	ja-dc	y		06-21
Asystasia gangetica (L.) T. And. ssp. *micrantha* (Nees) Ense.	Acanthaceae	gro (nat)	h	pe	3	da sg	ls	50	75	dc-mr	fb-ap	ja-dc	y		06-5
Hemigraphis griffithiana (Nees) T. And.	Acanthaceae	gro	h	pe	3	eg/bb	ls	50	75	ja-fb	fb-mr	ja-dc	y		06-29
Hygrophila phlomoides Nees	Acanthaceae	gro	h	a	3	wet areas in eg/bb	ls	50	100	dc-ja	ja-fb	my-fb	y		06-47
Justicia vasculosa (Wall. *ex* Nees) T. And.	Acanthaceae	epl (gro)	s (h) (l)	pd	3	rocks in eg/bb	ls	100	125	ja-mr	fb-ap	ja-dc	y		06-23
Pseuderanthemum graciliflorum (Nees) Ridl.	Acanthaceae	gro	l(h)	pe	3	eg/bb da sg	ls	50	75	dc-mr	fb-ap	ja-dc	y		06-69
Staurogyne merguensis O.K.	Acanthaceae	gro	h	pe	2	eg/bb	ls	25	75	sp-ja		ja-dc	y		05-522
Strobilanthes glaucescens Nees	Acanthaceae	gro	h	pd	3	streams in eg/bb	ls	50	75	dc-ja	ja-fb	ja-dc	y		06-7
Callicarpa arborea Roxb. var. *arborea*	Verbenaceae	gro	t	pe	3	da sg	ls ss	25	150	ag-oc	sp-oc	ja-dc	y	y	07-620
Clerodendrum infortunatum L.	Verbenaceae	gro	l(h)	pe	3	eg/bb da	ls	25	50		mr-ap	ja-dc		y	06-180
Clerodendrum paniculatum L.	Verbenaceae	gro	l	pe	2	eg/bb da	ls	50	100	jl-ag		ja-dc			06-539
Premna pyramidata Wall. *ex* Schau.	Verbenaceae	gro	t	pe	3	da sg	ss	25	150			ja-dc			
Vitex pinnata L.	Verbenaceae	gro	t	pd	3	eg/bb da sg	ls	25	75	sp-ja	ag	ja-dc	y	y	05-547, 06-582
Vitex quinata (Lour.) Will.	Verbenaceae	gro	t	pe	3	da sg	ls	25	75		sp-oc	ja-dc		y	05-558
Gomphostemma javanicum (Bl.) Bth.	Labiatae	gro	h	pe	2	streams in eg/bb	ls	50	75	ag-ja		ja-dc	y		06-11
Hyptis capitata Jacq.	Labiatae	gro	h	a	3	eg/bb da	ls	50	100	ja-fb	fb-mr	my-fb	y		06-45
Hyptis suaveolens (L.) Poit.	Labiatae	gro	h	a	3	da sg	ss	25	75	jl-sp	sp-oc	my-dc			
Pisonia umbellifera (J.R. & G. Forst.) Seem.	Nyctaginaceae	gro	t	pe	2	streams, ponds,wet areas	ls	50	100	sp-mr	mr-ap	ja-dc	y	y	05-509, 06-210

						in eg/bb									
Thottea tomentosa (Bl.) Hou	Aristolochiaceae	gro	h	pe	3	eg/bb	ls ss	25	100	jl-ag	ag-sp	ja-dc	y	y	06-542
Nepenthes mirabillis (Lour.) Druce	Nepentheaceae	gro	v	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75			ja-dc			
Chloranthus erectus (B.H.) Verd.	Chloranthaceae	gro	h	pe	3	eg/bb	ls	25	75	ja-fb	ag-fb	ja-dc		y	05-513
Endocomia macrocoma (Miq.) Wilde															
ssp. *prainii* (King) Wilde	Myristicaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Horsfieldia brachiata (King) Warb.	Myristicaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Horsfieldia irya (Gaertn.) Warb.	Myristicaceae	gro	t	pe	3	streams in eg/bb	ls	25	75	mr-ap	ag-sp	ja-dc	male		06-172
Knema andamanica (Warb.) Wilde															
ssp. *nicobarica* (Warb.) Wilde	Myristicaceae	gro	t	pe	2	eg/bb	ss	25	175		jl-ag	ja-dc			07-616
Cinnamomum iners Reinw ex. Bl.	Lauraceae	gro	t	pe	3	eg/bb	ls	25	75		mr-ap	ja-dc		y	06-173
Dehaasia longipetiolata Kosterm.	Lauraceae	gro	t	pe	3	eg/bb	ls	25	75	mr-ap		ja-dc	y		06-226
Litsea grandis (Wall. *ex* Nees) Hk.f.	Lauraceae	gro	t	pe	3	eg/bb	ls	25	50	mr-ap		ja-dc	y		06-222
Litsea umbellata (Lour.) Merr. var. *umbellata*	Lauraceae	gro	l	pe	3	da sg	ls	25	75	ag-oc	sp-nv	ja-dc	male	y	05-524, 06-189
Aquilaria malaccensis Lmk.	Thymeleaceae	gro	t	pe	3	eg/bb	ls	25	75			ja-dc			
Elytranthe albida (Bl.) Bl.	Loranthaceae	epi	s	pe	2	eg/bb	ls ss	25	175	ag-sp		ja-dc			
Macrosolen cochinchinensis (Lour.) Tiegh.	Loranthaceae	epi hemipar	s	pe	3	eg/bb da	ls	25	75		mr-ap	ja-dc		y	06-182
Scurrula parasitica L.	Loranthaceae	epi hemipar	s	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75	ag-oc		ja-dc	y		05-519
Dendrotrophe varians (Bl.) Miq.	Santalaceae	gro	wc	pe	3	streams, wet areas in eg/bb da	ls	25	75	dc-fb	fb-mr	ja-dc	y	y	06-32, 06-60
Sclerocarpum pentandrum (Denn.) Mabb.	Santalaceae	gro	t	pe	2	egf eg/bb	ls	50	75		ag	ja-dc			06-527
Champereia manillana (Bl.) Merr.	Opiliaceae	gro	l	pe	3	eg/bb da	ls	25	50		mr-ap	ja-dc		y	06-231
Lepionurus sylvestris Bl.	Opiliaceae	gro	l	pe	2	eg/bb	ls	50	100	mr-ap	mr-ap	ja-dc	y	y	06-230
Antidesma ghaesembilla Gaertn.	Euphorbiaceae	gro	t(l)	pe	3	da sg	ls ss	25	150		jl-sp	ja-dc			06-552
Antidesma leucopodium Miq.	Euphorbiaceae	gro	t	pe	3	eg/bb	ls	25	75	mr-ap		ja-dc	female	imm	06-225
Antidesma montanum Bl. var. *montanum*	Euphorbiaceae	gro	t(l)	pe	3	eg/bb	ls	25	100	mr-ap	ag	ja-dc	male,female	y	m06-171,fm06-237,fr06-531
Aporosa aurea Hk.f.	Euphorbiaceae	gro	t	pe	3	eg/bb	ls	50	125	ja-fb		ja-dc	female		06-35
Baccaurea motleyana (M. A.) M. A.	Euphorbiaceae	gro	t	pe	3	egf eg/bb	ss	25	175		ag-sp	ja-dc			s-24
Balakata baccata (Roxb.) Ess.	Euphorbiaceae	gro	t	pe	3	egf eg/bb	ss	75	150		jl-ag	ja-dc			
Breynia discigera M.A.	Euphorbiaceae	gro	l	pe	3	da sg	ls ss	25	100	ag-oc		ja-dc			06-584
Breynia vitis-ideae (Burm.f.) C.E.C. Fisc.	Euphorbiaceae	gro	l	pe	3	streams, wet areas in eg/bb	ls ss	50	100		ag-ja	ja-dc		y	06-16, 06-551
Bridelia tonentosa Bl.	Euphorbiaceae	gro,t	t,wc	pe	3	da/sg	ss	25	75	sp-ja	dc-fb	ja-dc			
Chaetocarpus castanocarpus (Roxb.) Thw.	Euphorbiaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75		mr-my	ja-dc		imm	06-190
Claoxylon longifolium (Bl.) Endl. *ex* Hassk.	Euphorbiaceae	gro	l	pe	2	eg/bb	ls	25	75	mr-ap		ja-dc	male		06-245
Croton griffithii Hk.f.	Euphorbiaceae	gro	t,l	pe	3	eg/bb da sg	ls	25	50	fb-mr	mr-ap	ja-dc	male	y	06-191
Elateriospermum tapos Bl.	Euphorbiaceae	gro	t	pe	2	egf eg/bb	ss	25	175		ag	ja-dc			s-87

Euphorbia antiquorum L.	Euphorbiaceae	epl	l	pd	2	rocks in eg/bb	ls	25	175			jn-fb			
Excoecaria cochinchinensis Lour.															
var. *viridils* (Pax & Hoffm.) Merr.	Euphorbiaceae	gro	l	pe	3	streams in eg/bb	ls	25	75	ag-mr	oc-ap	ja-dc	y	y	05-499
Galearia fulva (Tul.) Miq.	Euphorbiaceae	gro	t,l	pe	3	streams, wet areas in eg/bb	ls	25	75	mr-ap		ja-dc	male		06-198
Glochidion rubrum Bl.	Euphorbiaceae	gro	t(l)	pd (pe)	3	da sg	ls	25	100		ag-fb	ja-dc		y	05-537,06-68
Macaranga denticulata (Bl.) M.A.	Euphorbiaceae	gro	t	pe	3	da sg	ls ss	25	100			ja-dc			
Macaranga tanarius (L.) M.A. var. *tomentosa* (Bl.) M.A.	Euphorbiaceae	gro	t	pe	3	da sg	ls ss	25	100	ag-sp	sp-oc	ja-dc	male		07-619,07-622
Mallotus peltatus (Geisel.) M. A.	Euphorbiaceae	gro	l (s,t)	pe	3	eg/bb	ls	25	75	mr-ap		ja-dc	male		06-193
Phyllanthus albidiscus (Ridl.) A.S.	Euphorbiaceae	gro	s	pe	3	eg/bb	ls ss	25	100	ag	ja-fb	ja-dc	male	y	06-39,06-543
Phyllanthus oxyphyllus Miq.	Euphorbiaceae	gro	l	pe	3	wet areas in egf eg/bb da	ls ss	25	150	ag-sp		ja-dc	male		06-550
Phyllanthus pulcher Wall. *ex* M.A.	Euphorbiaceae	gro	s	pe	2	streams in eg/bb	ls	25	75	ag-oc		ja-dc			07-621
Phyllanthus reticulatus Poir.	Euphorbiaceae	gro	sc	pe	3	da sg	ss	75	150		ag-sp	ja-dc			06-589
Phyllanthus roseus (Craib & Hutch.) Beille	Euphorbiaceae	gro	l(s)	ped	3	da sg	ls	50	75	dc-ja		ja-dc	y		06-9
Phyllanthus urinaria L.	Euphorbiaceae	gro nat wee	h	a	3	da	ls	25	50	ja-my	ja-my	ja-dc	y	y	06-187
Sauropus androgynus (L.) Merr.	Euphorbiaceae	gro	l	pe	2	eg/bb	ss	25	75	jn-oc	sp-nv	ja-dc			07-613
Suregada multiflora (A. Juss.) Baill. var. *multiflora*	Euphorbiaceae	gro	t	pe	3	da sg	ss	50	200			ja-dc			
Triadica cochinchinensis Lour.	Euphorbiaceae	gro	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Trema orientalis (L.) Bl.	Ulmaceae	gro	t(l)	pe	3	da sg	ls ss	25	125	jl-ag		ja-dc	male		06-580
Artocarpus dadah Miq.	Moraceae	gro	t	pd	3	eg/bb da	ls	25	75	mr-ap	mr-ap	ja-dc	male	y	06-218
Artocarpus rigidus Bl.	Moraceae	gro	t	pe	2	egf eg/bb	ss	25	175			ja-dc			s-79
Ficus chartacea (Wall. *ex* Kurz) Wall. *ex* King	Moraceae	gro	l	pe	2	da sg	ls	25	75	ag-fb	ag-fb	ja-dc	y	y	05-536
Ficus (aff. *chartacea* (Wall. *ex* Kurz) Wall. *ex* King)	Moraceae	gro	t	pe	3	eg/bb	ls	25	75			ja-dc			
Ficus concinna (Miq.) Miq.	Moraceae	gro	t (l)	pe	3	streams, wet areas in eg/bb	ls	50	175	jl-sp	ag-oc	ja-dc			07-624
Ficus fistulosa Reinw. *ex* Bl.	Moraceae	gro	t	pe	3	eg/bb	ls	25	75	mr-my	ap-jn	ja-dc	y		06-211
Ficus globosa Bl.	Moraceae	gro	wc	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75	ag-oc	ag-oc	ja-dc	y	y	05-557
Ficus hirta Vahl	Moraceae	gro	l	pe	3	da sg	ss	25	150			ja-dc			
Ficus hispida L.f.	Moraceae	gro	t(l)	pe	3	da sg	ls ss	25	125	ja-dc	ja-dc	ja-dc			
Ficus microcarpa L.f.	Moraceae	gro epi epl str	t	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Ficus pisifera Voigt	Moraceae	gro	l sc	pe	2	streams, wet areas in eg/bb (swamp forest)	ls	25	75	ag-oc	ag-oc	ja-dc	y	y	05-548
Ficus sundaica Bl.	Moraceae	gro(epi)	t, wc	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75	ag-oc	ag-oc	ja-dc	y	y	05-560,06-533
Ficus tinctoria Forst. f. ssp. *gibbosa* (Bl.) Corn. var. *gibbosa*	Moraceae	gro	t	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75	ag-mr	sp-ap	ja-dc	y		05-507

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Ficus variegata Bl.	Moraceae	gro	t	pd	3	wet areas in eg/bb	ls	25	75	ag-mr	ag-ap	ja-dc	y		06-214
Streblus ilicifolius (Vidal) Corn.	Moraceae	gro	l(t)	pe	3	streams, wet areas in eg/bb	ls	50	125	mr-ap		ja-dc			
Dendrocnide stimulans (L.f.) Chew	Urticaceae	gro	t	pe	3	streams, wet areas in eg/bb	ls	25	75		mr-ap	ja-dc		y	06-209
Pellionia repens (Lour.) Merr.	Urticaceae	gro	cr	pe	3	eg/bb	ls	25	175	ag-sp		ja-dc	male		07-633
Poikilospermum suaveolens (Bl.) Merr.	Urticaceae	gro epi	wc	pe	3	wet areas in eg/bb	ls ss	25	125	mr-ap(ag)		ja-dc	male,female		06-208
Morella (Myrica) esculenta (B.-H.) Turn.	Myricaceae	gro	t	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75	oc-nv		ja-dc	male		05-562
Castanopsis schefferiana Hance	Fagaceae	gro	t	pe	3	eg/bb	ls	25	75		ag-sp	ja-dc		y	06-532
Lithocarpus falconeri (Kurz) Rehd.	Fagaceae	gro	t	pe	3	da sg	ls	50	100	sp-dc	mr-ap	ja-dc	female	y	06-58,06-181
ANGIOSPERMAE, MONOCOTYLEDONEAE															
Flagellaria indica L.	Flagellariaceae	gro	v	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75		sp-oc	ja-dc		y	05-517
Xyris indica L.	Xyridaceae	gro	h	a	3	wet areas in eg/bb	ls	50	75	nv-ja	ja-fb	jn-ja	y	y	06-65
Xyris pauciflora Willd.	Xyridaceae	aqu(gro)	h	pd(a)	3	wet areas in eg/bb	ls	50	75	nv-ja	dc-fb	jn-fb	y	y	06-66
Eriocaulon glabriflorum Ridl.	Eriocaulaceae	gro	h	a	3	eg/bb	ls	50	75	dc-fb	ja-mr	jn-mr	y	y	06-71
Eriocaulon truncatum B.-H. *ex* Mart.	Eriocaulaceae	aqu(gro)	h	a	3	wet areas in eg/bb	ls	50	75	dc-ja	ja-mr	jn-fb	y		06-70
Musa acuminata Colla ssp. *siame*a Simm.	Musaceae	gro	h	pe	3	da sg	ss	25	125	jl-sp	ag-oc	ja-dc			06-553
Amomum sp.	Zingiberaceae	gro	h	pe	3	egf eg/bb	ls ss	25	225			ja-dc			
Boesenbergia trangensis K. Lar.	Zingiberaceae	gro	h	pe	2	eg/bb	ls	25	75	sp-oc		ja-dc	y		05-527
Costus speciosus (Koen.) J.E. Sm. var. *speciosus*	Zingiberaceae	gro	h	pe	3	streams, wet areas in eg/bb da sg	ls ss	50	75	jl-ag	dc-ja	ja-dc		y	06-15
Curcuma aurantiaca van Zijp	Zingiberaceae	gro	h	pd	2	eg/bb	ss	25	100	ag-sp	oc-nv	my-ja			
Etlingera littoralis (Kon.) Gise.	Zingiberaceae	gro	h	pe	3	wet areas in eg/bb	ls	25	75	mr-ap		ja-dc			
Globba fasciata Ridl.	Zingiberaceae	gro	h	pe	3	da sg	ls	25	50	ag-oc	oc-nv	ja-dc	y		05-503
Zingiber zerumbet (L.) J.E. Sm.	Zingiberaceae	gro	h	pe	2	eg/bb	ls ss	25	125		ag-sp	ja-dc			
Donax cannaeformis (G. Forst.) K. Sch.	Marantaceae	gro	h	pe	3	wet areas in eg/bb	ls	25	75	mr-ap	ap-jn	ja-dc	y	imm	06-199
Peliosanthes teta Andr. ssp. *humilis* (Andr.) Jess.	Liliaceae	gro	h	pe	3	streams in eg/bb	ls	50	100		dc-ja	ja-dc		y	06-12
Dracaena curtisii Ridl.	Agavaceae	gro	h	pe	3	streams, wet areas in eg/bb	ls ss	25	75		ag-oc	ja-dc		y	05-521
Dracaena sp.	Agavaceae	gro	t	pe	2	eg/bb	ls ss	25	50			ja-dc			
Molineria latifolia (Dry. *ex* W.T. Ait.) Herb. *ex* Kurz	Amaryllidaceae, Hypoxidoideae	gro	h	pe	3	eg/bb	ls ss			jl-sp	sp-oc	ja-dc			06-562
Smilax blumei A.DC.	Smilacaceae	gro	v	pe	3	eg/bb da sg	ls	50	100	ja-fb	mr-ap	ja-dc	male		06-28
Aglaonema oblongifolium (Roxb.) Schott	Araceae	gro	h	pd	3	streams, wet areas in eg/bb	ls	25	75	sp-ja		ja-dc	y		05-526
Aglaonema simplex (Bl.) Bl.	Araceae	gro	h	pe	2	eg/bb da	ss	25	175	ag-sp	sp-oc	ja-dc			07-639
Alocasia denudata Engl.	Araceae	gro	h	pe	2	wet areas in eg/bb	ls	50	75		ja-fb	ja-dc		y	06-25

Amorphophallus paeoniifolius (Denn.) Nicol.	Araceae	gro	h	pd	2	rocks in eg/bb	ls	50	125		oc-nv	my-dc			
Arisaema roxburghii Kunth	Araceae	gro	h	pd	2	egf eg/bb	ls	50	100		ag-sp	my-dc		imm	06-535
Homalmena paludosa Hk.f.	Araceae	gro	h	pe	3	streams, wet areas in eg/bb	ls	25	75	sp-oc	nv-dc	ja-dc	y		05-510
Homalomena nutans Hk. f.	Araceae	gro	h	pe	3	streams in eg/bb	ls	25	150	sp-oc		ja-dc			07-609
Homalomena occulta (Lour.) Schott	Araceae	gro	h	pe	3	egf eg/bb	ss	25	175	jl-sp		ja-dc			07-636
Rhaphidophora gigantea (Schott) Ridl.	Araceae	gro	v	pe	3	streams, wet areas in eg/bb	ls	25	75	dc-mr		ja-dc	y		06-31
Rhaphidophora glauca (Wall.) Schott	Araceae	gro	v(cr)	pe	3	egf eg/bb	ls ss			oc-dc		ja-dc			
Rhaphidophora peepla (Roxb.) Schott	Araceae	gro	v(cr)	pe	3	eg/bb, swamp forest	ls	50	75			ja-dc			
Typhonium trilobatum (L.) Schott	Araceae	gro (wee)	h	pd	3	da	ss	25	75			my-dc			
Stemona curtisii Hk.f.	Stemonaceae	gro	v	pe	3	streams in da sg	ls	50	75	ja-mr		ja-dc	y		06-18
Dioscorea oryzetorum Pr. & Burk. var. *oryzetorum*	Dioscoreaceae	gro	v	pe	3	da/sg	ss	25	75	dc-ja	ja-fb	ja-dc			
Calamus axillaris Becc.	Palmae	gro	wc	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Calamus exilis Griff.	Palmae	gro	wc	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Calamus javensis Bl.	Palmae	gro	wc	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Calamus palustris Griff. var. *cochinchinensis* Becc.	Palmae	gro	wc v	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Caryota maxima Bl.	Palmae	gro	t	pe	3	wet areas in eg/bb, egf	ls ss	25	150	mr-sp	mr-sp	ja-dc	y	y	06-197
Daemonorops sabut Becc.	Palmae	gro	wc	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Korthalsia laciniosa (Griff.) Mart.	Palmae	gro	wc	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Licuala kunstleri Becc.	Palmae	gro	l	pe	3	streams, wet areas in eg/bb	ls	25	100	ja-fb		ja-dc	y		06-37
*Onchosperma tigillatrum (*Jack) Ridl.	Palmae	gro	t	pe	2	streams in eg/bb	ls	25	75			ja-dc			
Orania sylvicola (Griff.) H.E. Moore	Palmae	gro	t	pe	3	egf eg/bb	ss	50	225			ja-dc			
Pinanga malaiana (Mart.) Scheff.	Palmae	gro	t	pe	3	wet areas in eg/bb	ls	25	75		mr-ap	ja-dc		y	06-233
Salacca wallichiana Mart.	Palmae	gro	l	pe	4	streams, wet areas in eg/bb	ls ss	25	125			ja-dc			
Freycinetia sumatrana Hemsl. var. *sumatrana*	Pandanaceae	gro	v(cr)	pe	3	streams, wet areas in eg/bb	ls	25	75			ja-dc			
Pandanus (*unicornutus* St. John)	Pandanaceae	gro	t	pe	2	streams, wet areas in eg/bb	ls	50	75			ja-dc			
Pandanus ovatus (Gaud.) Kurz	Pandanaceae	gro	l(h)	pe	3	eg/bb da sg	ls ss	25	125		mr-ap	ja-dc		y	06-239
Tacca chantrieri Andre	Taccaceae	gro	h	pe	3	streams, wet areas in eg/bb	ls	50	100	ag-fb	ja-mr	ja-dc	y		06-52
Tacca palmata Bl.	Taccaceae	gro	h	pe	3	eg/bb	ls	50	75		fb-sp	ja-dc			
Aphyllorchis pallida Bl.	Orchidaceae	gro sap	h	pd	2	egf	ss	200	225	jl-ag	ag-sp	leafless			06-578
Apostasia wallichii R.Br.	Orchidaceae	gro	h	pd	2	streams in eg/bb	ls	25	75		sp-oc	ja-dc		y	05-523
Arundina graminifolia (D.Don) Hochr.	Orchidaceae	gro	h	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75	ag-ja		ja-dc	y		05-563
Calanthe ceciliae Rchb. f.	Orchidaceae	gro	h	pe	1	wet areas in eg/bb, swamp	ls	75	100	ag		ja-dc			06-536

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						forest									
Calanthe rubens Ridl.	Orchidaceae	gro	h	pd	2	streams, wet areas in eg/bb	ls	50	100	ja-fb		jn-dc	y		06-76
Corymborkis veratrifolia (Reinw.) Bl.	Orchidaceae	gro	h	pe	2	eg/bb, swamp forest	ls	25	75			ja-dc			
Cymbidium sp.	Orchidaceae	epi	h	pe	3	streams, wet areas in eg/bb, swamp forest	ls	25	75			ja-dc			
Dendrobium secundum (Bl.) Lindl.	Orchidaceae	epi	h	pe(pd)	3	streams, in eg/bb	ls	25	75	mr-ap		ja-dc	y		06-184
Habenaria limprichitii Schltr.	Orchidaceae	gro	h	pd	2	eg/bb	ls	25	75	ag		my-dc			06-567
Nervilia aragoana Gaud.	Orchidaceae	gro	h	pd	3	streams, wet areas in eg/bb	ls	25	75	mr-ap		my-dc	y		05-530,06-206
Plocoglottis lowii Rchb.f.	Orchidaceae	gro	h	pe	2	streams, wet areas in eg/bb	ls	50	100	ja-fb	fb-mr	ja-dc	y		06-38
Carex indica L. var. *indica*	Cyperaceae	gro	h	pe	3	da sg	ls	25	50	fb-my	fb-my	ja-dc	y	y	06-236
Cyperus kyllingia Endl.	Cyperaceae	wee(gro)	h	pe	3	da sg	ls	25	50	ja-dc	ja-dc	ja-dc	y	y	06-238
Diplacrum caricinum R.Br.	Cyperaceae	gro	h	pe	3	wet areas in eg/bb	ls	50	75	dc-ja	ja-fb	ja-dc	y		06-27
Fimbristylis cymosa R.Br.	Cyperaceae	gro	h	pe	4	streams, ponds,wet areas in eg/bb	ls	25	75	ag-oc	sp-nv	ja-dc	y		05-505
Fimbristylis dichotoma (L.) Vahl ssp. *dichotoma*	Cyperaceae	gro	h	pe	3	da sg	ls ss	25	75	jl-oc	jl-oc	ja-dc			06-559
Fimbristylis disticha Boeck.	Cyperaceae	gro	h	a	3	da	ss	25	75	jl-oc	sp-nv	my-ja			07-623
Hypolytrum nemorum (Vahl) Spreng. var. *nemorum*	Cyperaceae	gro	h	pe	3	da sg	ls	50	75	ja-fb	mr-ap	ja-dc	y		06-79
Rhynchospora corymbosa (L.) Britt.	Cyperaceae	gro(aqu)	h	pe	3	ponds, wet areas	ss	25	75	jn-oc	jl-nv	ja-dc			07-638
Scleria purpurascens Steud. var. *purpurascens*	Cyperaceae	gro	h	pe	3	da sg	ls ss	25	100	jl-sp	ag-oc	ja-dc			06-560
Scleria scrobiculata Nees & Mey. *ex* Nees ssp. *scrobiculata*	Cyperaceae	gro	h	a	3	da sg	ls	25	50	jl-nv	ag-dc	ja-dc	y	y	05-502,06-566
Scleria terrestris (L.) Fass.	Cyperaceae	gro	h	pe	3	streams, ponds,wet areas in eg/bb	ls ss	25	75	jl-sp	sp-nv	ja-dc	y	y	05-528,06-564
Acroceras tonkinense (Balan.) C.E. Hubb. *ex* Bor	Gramineae	gro	h	pe		da sg	ls ss	25	75	jl-sp	ag-oc	ja-dc			06-565
Axonopus compressus (Swz.) P. Beauv.	Gramineae	gro(wee)	h	pe	3	streams, wet areas in eg/bb da	ls ss	25	175	jl-oc	ag-nv	ja-dc			07-625
Centotheca lappacea (L.) Desv. var. *lappacea*	Gramineae	gro	h	a(pe)	3	da sg	ls	50	75	dc-ja	ja-fb	ja-dc	y		06-8
Cynodon dactylon (L.) Pers.	Gramineae	gro(wee)	h	pe	3	streams, wet areas in eg/bb da	ls ss	25	175	jl-oc	ag-nv	ja-dc			07-626
Cyrtococcum oxyphyllum (Steud.) Stapf	Gramineae	gro	h	pe	3	da sg	ss	25	125	jl-sp	jl-sp	ja-dc			06-574
Eragrostis pilosa (L.) P. Beauv.	Gramineae	gro wee	h	a	3	streams, ponds,wet areas in eg/bb	ls	25	75	ag-oc	ag-oc	my-nv	y		05-532
Eragrostis unioloides (Retz.) Nees *ex* Steud.	Gramineae	gro	h	pe	3	da sg	ls ss	25	75	ag-oc	ag-oc	ja-dc			06-591
Imperata cylindrica (L.) P. Beauv.															
var. *major* (Nees) C.E. Hubb. *ex* Hubb. & Vaugh.	Gramineae	gro	h	pe	5	da sg	ls ss	25	150	ag-oc	sp-nv	ja-dc			
Oryza meyeriana (Zoll. & Mor.) Baill.															

var. *granulata* (Watt) Duist.	Gramineae	gro	h	pd(pe)	3	streams in eg/bb	ls	50	75	nv-dc	ja-fb	ja-dc	y	y	06-10
Ottochloa nodosa (Kunth) Dandy	Gramineae	gro	h	pd	3	da	ls	25	50	jn-nv	jl-dc	ap-dc	y	y	05-501
Phragmites vallatoria (Pluk. ex L.) Veldk.	Gramineae	gro	h	pe	3	da sg	ls ss	25	150	ag-oc	ag-oc	ja-dc			
Thysanolaena latifolia (Roxb. *ex* Horn.) Honda	Gramineae	gro	h	pe	4	da sg	ls ss	25	150	sp-nv	oc-dc	ja-dc			
Cephalostachyum virgatum (Munro) Kurz	Gramineae, Bambusoideae	gro	h	pe	4	streams, ponds,wet areas in eg/bb	ls	25	75	jl-nv	ag-dc	ja-dc	y		05-559
Dinochloa scandens (Bl.) O.K.	Gramineae, Bambusoideae	gro	h	pe	3	eg/bb	ls	25	75			ja-dc			
Gigantochloa apus (Schult.) Kurz	Gramineae, Bambusoideae	gro	s(h)	pe	4	wet areas in eg/bb	ls	25	75			ja-dc			
Gigantochloa nigrociliata (Buse) Kurz	Gramineae, Bambusoideae	gro	h	pe	3	eg/bb	ls ss	25	125	jl-sp	ag-sp	ja-dc	y		06-530, 07-615
Gigantochloa wrayi Gamb.	Gramineae, Bambusoideae	gro	h	pe	3	wet areas in eg/bb	ls	25	75			ja-dc			
Thyrostachys oliveri Gamb.	Gramineae, Bambusoideae	gro cul	s(h)	pe	3	eg/bb	ls	25	75			ja-dc			
GYMNOSPERMEAE															
Gnetum microcarpum Bl.	Gnetaceae	gro	wc	pe	3	eg/bb	ls	50	100	ja-fb		ja-dc	male		06-30
PTERIDOPHYTA															
Lycopodium cernuum L.	Lycopodiaceae	gro	h	pe	3	wet areas in eg/bb da sg	ls	50	100	mr-ap		ja-dc	y		06-229
Lycopodium phlegmaria L.	Lycopodiaceae	epi	h	pe	2	eg/bb, swamp forest	ls	25	75	ag-oc	ag-oc	ja-dc			
Selaginella repanda (Desv.) Spring	Selaginellaceae	gro epl	h	a	3	da sg	ls ss	25	125	ag-oc	ag-oc	my-nv	y	y	05-535, 06-548
Selaginella willdenowii (Desv.*ex* Poir.) Bak.	Selaginellaceae	gro	v(h)	pe	4	da sg	ls	25	75	ja-dc	ja-dc	ja-dc	y	y	05-552
Dicranopteris linearis (Burm.f.) Underw. var. *linearis*	Gleicheniaceae	gro	h	ped	3	eg/bb da sg	ls	50	100	dc-fb	dc-fb	ja-dc	y		06-32
Lygodium flexuosum (L.) Sw.	Schizaeaceae	gro	v	pe	3	da sg	ls ss	25	150			ja-dc			
Lygodium microphyllum (Cav.) R. Br.	Schizaeaceae	gro	v	pe	3	da sg	ls ss	50	100	nv-fb	nv-fb	ja-dc	y		06-78
Lygodium polystachyum Wall. *ex* Moore	Schizaeaceae	gro	v	pe	3	da sg	ss	25	125	ag-oc	ag-oc	ja-dc			06-579
Schizaea digitata (L.) Sw.	Schizaeaceae	gro	h	pe	2	streams, ponds,wet areas in eg/bb	ls	50	75	ag-mr	ag-mr	ja-dc	y	y	05-529
Pteridium aquilinium (L.) Kuhn ssp. *caudatum* (L.)															
Tag. & K. Iw. var. *yarrabense* Dom.	Dennstaedtiaceae	gro wee	h	pe	3	da sg	ss	75	225			ja-dc			
Davallia divarioata Bl.	Davalliaceae	epi	h	pe	3	egf eg/bb	ls ss	25	125	jl-sp	jl-sp	ja-dc			06-583
Nephrolepis biserrata (Sw.) Schott	Oleandraceae	gro	h	pe	3	streams, wet areas in eg/bb	ls	25	75	ja-dc	ja-dc	ja-dc	y	y	05-550
Adiantum flabellulatum L.	Parkeriaceae	gro	h	pe	2	da sg	ss	75	125	ag-sp	ag-sp	ja-dc			06-558
Cheilanthes belangeri (Bory) C. Chr.	Parkeriaceae	gro	h	pd	3	da sg	ss	25	150	jl-sp	jl-sp	my-dc			06-549
Hemionitis arifolia (Burm. f.) Moore	Parkeriaceae	gro	h	pe	2	da sg	ss	25	125	ag-sp	ag-sp	ja-dc			06-556
Taenitis blechnoides (Willd.) Sw.	Parkeriaceae	gro	h	pe	3	egf eg/bb	ss	75	225	ag-oc	ag-oc	ja-dc			06-575
Vaginularia paradoxa (Fee) Mett.	Vittariaceae	epi	h	pe	2	egf	ss	150	225	ag-oc	ag-oc	ja-dc			06-576
Vittaria angustifolia Bl.	Vittariaceae	epi	h	pe	3	da	ss	25	75	ja-dc	ja-dc	ja-dc	sori		07-611
Acrostichium aureum L.	Pteridaceae	gro	h	pe	2	streams, wet areas in eg/bb,	ls	25	75			ja-dc			

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						swamp forest									
Pteris ensiformis Burm. f.	Pteridaceae	gro	h	pe	3	eg/bb	ls	25	175	jl-oc	jl-oc	ja-dc	sori		07-627
Pteris vittata L.	Pteridaceae	gro epi epl	h	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75	ja-dc	ja-dc	ja-dc	y	y	05-543
Stenochlaena palustris (Burm. f.) Bedd.	Pteridaceae	gro	v	pe	3	streams, wet areas in eg/bb, swamp forest	ls	25	75			ja-dc			
Asplenium nidus L. var. *nidus*	Aspleniaceae	epi	h	pe	2	egf eg/bb	ls ss	75	225	jl-oc	jl-oc	ja-dc			
Heterogonium pinnatum (Copel.) Holtt.	Dryopteridaceae	gro	h	pe	2	egf eg/bb	ls	75	100	jl-sp	jl-sp	ja-dc	y		06-537
Tectaria angulata (Willd.) C. Chr.	Dryopteridaceae	gro	h	pe	3	streams in eg/bb	ls ss	50	100	nv-fb	nv-fb	ja-dc	y		06-63
Tectaria maniliensis (Presl) Holtt.	Dryopteridaceae	epl	h	pd	2	rocks in egf eg/bb	ls	75	100	ag-sp	ag-sp	jn-dc			06-538
Thelypteris immersa (Bl.) Ching	Thelypteridaceae	gro	h	pe	3	streams, ponds,wet areas in eg/bb	ls	25	75	jl-oc	jl-oc	ja-dc	y	y	05-561
Thelypteris interrupta (Willd.) K. Iw.	Thelypteridaceae	gro	h	pe	3	ponds, wet ares in eg/bb	ss	25	75	jl-fb	jl-fb	ja-dc	sori		07-637
Thelypteris terminans (Hk.) Tag. & K. lw.	Thelypteridaceae	gro	h cr	pe	3	streams, wet areas in eg/bb	ls	25	75	ag-oc	ag-oc	ja-dc	y	y	05-515, 06-563
Aglaomorpha coronans (Wall. *ex* Mett.) Copel.	Polypodiaceae	epi	h	pe	3	egf eg/bb	ls	25	75			ja-dc			
Drynaria queroifolia (L.) J. Sm.	Polypodiaceae	epi	h	pe	3	egf eg/bb	ls ss	25	150	jl-oc	jl-oc	ja-dc			06-569
Microsorum punctatum (L.) Copel.	Polypodiaceae	epi	h	pe	3	eg/bb sg	ls ss	25	150	ag-oc	ag-oc	ja-dc			06-568
Microsorum scolopendria (Burm. f.) Copel.	Polypodiaceae	gro	h	pe	3	streams, ponds,wet areas in eg/bb	ls	50	75	ag-nv	ag-nv	ja-dc	y	y	05-506
Platycerium coronarium (Koen.) Desv.	Polypodiaceae	epi	h	pe	2	eg/bb	ls ss	25	175			ja-dc			

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Full Paper

Using chemometrics in assessing Langat River water quality and designing a cost-effective water sampling strategy

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Abstract: Seasonally dependent water quality data of Langat River was investigated during the period of December 2001 – May 2002, when twenty-four monthly samples were collected from four different plots containing up to 17 stations. For each sample, sixteen physico-chemical parameters were measured in situ. Multivariate treatments using cluster analysis, principal component analysis and factorial design were employed, in which the data were characterised as a function of season and sampling site, thus enabling significant discriminating factors to be discovered. Cluster analysis study based on data which were characterised as a function of sampling sites showed that at a chord distance of 75.25 two clusters are formed. Cluster I consists of 6 samples while Cluster II consists of 18 samples. The sampling plots from which these samples were taken are readily identified and the two clusters are discussed in terms of data variability. In addition, varimax rotations of principal components, which result in varimax factors, were used in interpreting the sources of pollution within the area. The work demonstrates the importance of historical data, if they are available, in planning sampling strategies to achieve desired research objectives, as well as to highlight the possibility of determining the optimum number of sampling stations which in turn would reduce cost and time of sampling.

Keywords: chemometrics, principal component analysis, cluster analysis, factorial design

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Introduction

Environmental data may be highly complex and depend on unpredictable factors that are usually characterised by their high variability. The main origins of this variability are geogenic, hydrological, meteorological and also anthropogenic (such as different emitters and dischargers) [1]. Due to the non-linear nature of environmental data, analysing these data may be tricky. The multivariate nature of these data together with their complex interrelation requires that multivariate data analysis techniques be employed in order to decipher any structure within the data. In this study, chemometric methods were used to determine sampling sites which are significantly different from each other. This work is motivated by the fact that an understanding of the nature of these sites would help in reducing the number of redundant sampling sites, thus reducing cost and time.

The data selected in this study came from 4 different sampling plots which in turn include 17 sampling sites. The selected plots, namely Kampung Bukit Dugang, Kampung Jenderam, Bukit Changgang and Labohan Dagang are located along the Langat River and are dominated by palm oil activities. Originally, the sampling plots were identified based on the economic needs of two particular districts involved in this study area (Kuala Langat and Sepang Districts). The main economic activities for both districts are agriculture and industry with palm oil plantation as the main agricultural activity.

The Langat River Basin is one of the most studied river basins in Malaysia. A respectable amount of secondary data is available from past research which can be used to obtain much information to help us in designing new studies of the Langat River Basin. This has motivated us to carry out this chemometric work.

Chemometrics can be considered as a branch of analytical chemistry which mainly uses multivariate statistical modeling in data treatment [2]. Massart et al. [3] defined chemometrics as 'a chemical discipline that uses mathematics, statistics, and formal logic; (a) to design or select optimal experimental procedures, (b) to provide maximum relevant chemical data, and (c) to obtain knowledge about chemical systems.' Chemometric methods have also been used for the classification and comparison of different samples [3]. It is also mentioned as the best approach to avoid misinterpretation of a large complex environmental monitoring data [2]. The application of chemometric to monitoring data makes it possible to compare this data with data on similar natural water sources in order to obtain a complete overview of the Langat River water quality. Among examples of the use of chemometrics are as a multicriteria decision-making [4], investigation of variable and site correlations [5] as well as determination of correlation of chemical and sensory data in drinking water [6]. Its applications in evaluating environmental data have also been demonstrated earlier by other researchers [7-9]. Chemometric methods have also been widely used as a tool in unsupervised pattern recognition of water quality data to draw out meaningful information. Chemometric methods have often been used in exploratory data analysis for the classification of different samples (observations) or sampling stations [8,10] and the identification of pollution sources[3,11,12]. The method have also been applied to characterise and evaluate the surface and freshwater quality as well as verifying their spatial and temporal variations caused by natural and anthropogenic factors based on seasonality [13,14]. Over the decades, use of chemometrics as a

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pattern recognition method have become an important tool in environmental sciences [15,16] to reveal and evaluate complex relationships in a wide variety of environmental applications [17]. The most common method of chemometrics used is to study clustering of data. In this respect, hierarchical agglomerative cluster analysis (HACA), principal components analysis (PCA) and factor analysis (FA) [18] are commonly employed. The applications of different pattern recognition techniques to reduce the complexity of large data sets have also been observed to achieve a better interpretation and understanding of water quality [19].

This study was carried out to fulfill three main objectives: (i) to apply chemometrics in recognising patterns in the sampling data, thus enabling researchers to determine effective sampling sites based on specific needs, (ii) to assess the water quality of Langat River and generally determine its sources of pollution, and (iii) to encourage the use of secondary data to help scientists and researchers design better approaches for future studies.

Materials and Methods

Study site

Langat River Basin is formed by three main rivers, which are the Langat River, Semenyih River and Labu River. The rivers flow across the states of Negeri Sembilan and Selangor for a distance of 125.6 km. Langat River is one of the most important raw water resources for drinking water and other activities such as recreation, industry, fishery and agriculture. In this area, agriculture is the main activity and covers 53.1% of the area, while 3.6% are for commercial purposes. Palm oil plantation takes 20,993 ha from the area and another 13,574 ha is covered by rubber plantation.

Seventeen sampling sites were selected in this study (see Table 1). Previously, the justification for selecting the location of these sampling stations was based on the economic activities of the selected areas. The sampling stations are divided into four plots; plots one and two, namely Kampung Bukit Dugang and Kampung Jenderam, covers five sampling stations located in the Sepang District. Plots three and four, namely Bukit Changgang and Labohan Dagang, are located in the Kuala Langat District consisting of four and three sampling stations respectively (see Table 1).

Data source

The data for this study was kindly provided to us by the Institute for Environment and Development (LESTARI), University Kebangsaan Malaysia. The data consists of 102 observations collected from all plots (consisting of 17 sampling stations) between December 2001 and May 2002. The sampling dates were set to coincide with two weather conditions: three observations in dry weather season (10th January 2002, 19th February 2002 and 15th May 2002) and another three during the rainy season (26th December 2001, 3rd March 2002 and 13th April 2002). Table 2 shows the stations sampled during each site visit. Based on these previous studies carried out by LESTARI,

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Table 1. Locations of plots and sampling stations

District	Study area (plot no.)	Station no.	Coordinate		Area description
			Latitude	Longitude	
Sepang	Kampung Bukit Dugang (Plot 1)	1.1	101°43.387'	02°53.778'	• Surrounded by palm oil plantation • Orangasli village • Sand mining (st. 1.4 & 1.5)
		1.2	101°43.282'	02°53.904'	
		1.3	101°43.262'	02°53.818'	
		1.4	101°43.088'	02°53.760'	
		1.5	101°42.925'	02°53.633'	
	Kampung Jenderam (Plot 2)	2.1	101°43.853'	02°52.036'	• Surrounded by palm oil plantation • Village
		2.2	101°43.523'	02°52.177'	
		2.3	101°43.208'	02°52.430'	
		2.4	101°42.795'	02°52.841'	
		2.5	101°42.571'	02°53.013'	
Kuala Langat	Bukit Changgang (Plot 3)	3.1	101°39.079'	02°49.156'	• Surrounded by palm oil plantation • Village
		3.2	101°38.590'	02°48.806'	
		3.3	101°38.564'	02°48.823'	
		3.4	101°38.500'	02°48.787'	
	Labohan Dagang (Plot 4)	4.1	101°36.990'	02°47.510'	• Surrounded by palm oil plantation • Village • Wetland (st. 4.3)
		4.2	101°36.964'	02°47.520'	
		4.3	101°36.853'	02°47.454'	

sixteen physicochemical properties of the water were determined: temperature, pH, TSS, DO, BOD, COD, conductivity, ammonical nitrogen (AN), nitrate, sulphate, phosphate, lead, cadmium, iron, zinc and copper content (Table 3). We used these secondary data for our work.

Statistical procedures

Twenty-four samples were selected out of 102 samples using the 90th percentile method for each sampling site on the same sampling date. These 90th percentile values were then compiled consistent with the standard table template. The whole process of manipulation and calculation of the 90th percentile values was carried out employing PHStat for Excel 97 & 2000 package [18].

In this study HACA was employed to investigate the group sampling sites (spatial) for the study regions [20]. HACA is a common method to classify [21] the variables or cases (observations/samples) into classes (clusters) with high homogeneity level within a class and high heterogeneity level between classes with respect to a predetermined selection criterion [22]. Ward's method, using Euclidean distances as a measure of similarity [23-25] with standardised data, is usually applied in HACA as a very efficient method and the result is illustrated by a dendogram of the groups and their proximity [26]. The Euclidean distance (linkage distance), reported as D_{link}/D_{max},

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Table 2. Weather conditions under which samples were taken

Plot	Station	Sampling date					
		a	**b**	**c**	**d**	**e**	**f**
	1.1	cloudy	cloudy	dry	overcast	overcast	overcast
	1.2	cloudy	dry	dry	overcast	overcast	clear
I	1.3	cloudy	dry	dry	overcast	overcast	clear
	1.4	overcast	dry	dry	overcast	overcast	clear
	1.5	overcast	dry	dry	overcast	overcast	clear
	2.1	overcast	dry	dry	overcast	overcast	dry
	2.2	overcast	dry	dry	overcast	overcast	dry
II	2.3	overcast	dry	dry	overcast	overcast	dry
	2.4	overcast	dry	dry	overcast	overcast	dry
	2.5	overcast	dry	dry	overcast	overcast	dry
	3.1	overcast	dry	dry	overcast	overcast	dry
III	3.2	overcast	dry	dry	overcast	overcast	dry
	3.3	overcast	dry	dry	overcast	overcast	dry
	3.4	overcast	dry	dry	overcast	clear	dry
	4.1	overcast	dry	dry	overcast	clear	dry
IV	4.2	overcast	dry	dry	overcast	clear	dry
	4.3	overcast	dry	dry	overcast	clear	dry

Note: (a) 26 December 2001, (b) 10 January 2002, (c) 19 February 2002, (d) 3 March 2002, (e) 13 April 2002 and (f) 15 May 2002

which represents the quotient between the linkage distance for a particular case divided by the maximal distance, is used, multiplied by 100 as a way to standardise the linkage distance represented by the *y*-axis [14,12,27].

The most powerful chemometric technique which is usually coupled with HACA is the PCA. It provides information on the most significant parameters due to spatial and temporal variations, which describe the whole data set excluding the less significant parameters with minimum loss of original information [14,27,28]. The PC can be expressed as:

$$z_{ij} = a_{i1}x_{1j} + a_{i2}x_{2j} + \ldots + a_{im}x_{mj} \qquad (1)$$

where z is the component score, a is the component loading, x the measured value of variable, I is the component number, j is the sample number, and m is the total number of variables.

Eigenanalysis of the sampled data was performed to extract the principal components (PCs) of the measured data using two selection criteria, i.e. the scree plot test and the corrected average eigenvalue. PCs with eigenvalues more than 1 are considered significant [28] in obtaining new groups of variables. Hierarchical cluster analysis was also employed in this study. In cluster analysis (CA), the squared Euclidean distance between normalised data was used to measure similarities between

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samples. Both average linkage between groups and Ward's method were applied to the standardised data and the results obtained were represented as dendograms. Two-factor factorial designs [29,30] were employed to identify the effect of season on the water quality.

The PCs generated by PCA are sometimes not readily interpreted. Therefore, it is advisable to rotate the PCs by varimax rotation. Varimax rotations applied on the PCs with eigenvalues more than 1 are considered significant [28] in order to obtain new groups of variables called varimax factors (VFs). The number of VFs obtained by varimax rotations is equal to the number of variables in accordance with common features and can include unobservable, hypothetical, and latent variables [11]. VF coefficients having a correlation greater than 0.75, between 0.75 - 0.50, and between 0.50 - 0.30 are considered as 'strong', 'moderate', and 'weak' significant factor loading respectively [31]. In this study, VF coefficients that show strong significant factor loadings will be discussed. Source identification of different pollutants is based on the different activities in the catchment area in light of previous literature.

Results and Discussion

Table 3 shows selected data obtained from the samples collected. Out of the 102 samples available, 24 samples from the four different plots were selected for this study. The choice of 24 samples was made to cover all possible weather conditions while the number of redundant samples was reduced. Plots 1 and 2 consist of five sampling sites each. Plot 3 consists of four sampling sites and plot 4 consists of three sampling sites. These selected samples were collected in six different sampling days and for each of the 24 samples, 16 features were evaluated.

Cluster analysis

Cluster analysis is a common method applied in unsupervised pattern recognition [1,32]. It was applied in this work to search for clusters due to different sampling seasons or different sampling sites by using water quality variables or features. The agglomerative hierarchical cluster analysis according to Ward's methods [21,23] using squared Euclidean distances was applied to detect multivariate similarities between sampling sites in different sampling plots at different sampling days. From Figure 1 it is observed that the separation between clusters 1 and 2 does not show a significant impact due to seasonal change. Differences in the feature values (water quality parameters) are probably due to seasonal changes distributed over the whole area of sampling plots. They do not, however, form the basis for the separation observed in the objects (sampling sites).

On the other hand, Figure 2 shows that if the separation is grouped according to sampling plots, it shows clear discrimination of Labohan Dagang and the other sites. It can be seen that Labohan Dagang (Group 1) sampling plot at similarity level 75.25 (dashed line in Figure 2) is very different from the others. In this study the other sampling plots that merge at similarity level 75.25 (Bukit Changgang, Kampung Jenderam and Kampung Bukit Dugang) forms a single group (Group 2).

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Table 3. Physicochemical properties of water at various sampling sites

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sampling site	pH	Temp. (°C)	Cond. (µS/cm)	TSS (mg/L)	DO (mg/L)	BOD (mg/L)	COD (mg/L)	AN (mg/L)	PO_4 (mg/L)	NO_3 (mg/L)	SO_4 (mg/L)	Pb (mg/L)	Cd (mg/L)	Fe (mg/L)	Zn (mg/L)	Cu (mg/L)
Kampung Bukit Dugang (26/12/2001)	5.8	30.0	69	65.4	3.0	5.44	21	1.57	0.16	3.1	0.8	0.54	0.01	2.8	0.04	32.56
Kampung Jenderam (26/12/2001)	3.5	27.0	126	2.8	1.5	3.74	18	1.57	0.14	0.9	6.6	0.26	0.01	2.2	0.08	2.01
Bukit Changgang (26/12/2001)	5.9	28.0	67	186.3	4.7	6.20	9	1.32	0.08	1.3	0.6	0.37	0.01	0.09	0.02	2.46
Labohan Dagang (26/12/2001)	5.8	29.0	96	815.3	3.6	5.44	45	0.57	0.04	6.3	138.9	0.55	0.02	2.2	0.08	2
Kampung Bukit Dugang (10/01/2002)	5.8	32.0	74	10.6	4.3	2.00	9	1.60	1.50	2.6	3.0	1.65	0.15	2.44	2.28	2.92
Kampung Jenderam (10/01/2002)	5.2	24.5	211	1.6	1.2	0.45	6	2.41	0.85	0.8	15.9	3.42	0.44	1.46	2.04	2.41
Bukit Changgang (10/01/2002)	5.3	29.6	189	283.7	4.2	1.32	24	1.34	0.11	2.8	20.6	2.73	0.14	3.8	2.24	3.31
Labohan Dagang (10/01/2002)	5.6	30.0	175	746.9	1.7	0.68	10	0.87	0.03	5.7	102.6	1.11	0.16	0.38	1.67	2.05
Kampung Bukit Dugang (19/02/2002)	5.5	31.0	76	95.4	4.2	2.51	8	1.24	1.94	1.4	2.0	3.85	0.25	2.59	2.19	71.95
Kampung Jenderam (19/02/2002)	6.3	28.1	255	0.1	0.3	0.10	1	2.22	0.96	0.7	13.0	4.28	0.45	1.61	1.88	2.38
Bukit Changgang (19/02/2002)	5.4	32.9	215	119.9	5.0	1.17	2	1.71	0.12	3.9	25.0	2.57	0.13	5.87	1.96	1.44
Labohan Dagang (19/02/2002)	5.5	30.5	290	724.3	0.6	0.01	27	1.44	0.01	3.9	44.0	1.79	0.13	0.62	2.23	1.62
Kampung Bukit Dugang (3/03/2002)	5.7	30.5	29	158.9	4.2	1.28	7	0.60	0.01	0.9	7.0	8.27	0.67	1.92	3.96	0.49
Kampung Jenderam (3/03/2002)	4.7	28.2	105	0.1	1.2	1.63	25	1.95	0.04	0.8	7.0	6.85	0.36	0.81	3.6	0.19
Bukit Changgang (3/03/2002)	4.2	29.2	153	147.6	1.1	1.14	0	1.84	0.01	1.4	27.0	3.57	0.69	3.47	3.42	0.26
Labohan Dagang (3/03/2002)	5.1	29.1	74	951.4	3.4	0.29	10	2.04	0.01	1.1	31.0	2.84	0.18	0.16	5.89	0.12
Kampung Bukit Dugang (13/04/2002)	5.8	29.4	76	188.1	2.3	0.50	8	0.50	0.14	1.1	5.0	4.45	0.39	1.27	3.41	0.12
Kampung Jenderam (13/04/2002)	5.2	29.6	106	0.2	2.1	0.43	1	1.89	0.26	1	1.0	2.58	0.18	1.18	6.87	0.13
Bukit Changgang (13/04/2002)	5.9	29.8	132	123.5	3.6	0.99	2	1.89	0.01	1.5	32.0	2.39	0.43	3.21	3.14	0.03
Labohan Dagang (13/04/2002)	5.1	29.9	92	795.7	4.0	0.67	26	1.99	0.01	1.2	29.0	3.81	0.1	0.14	7.21	0.18
Kampung Bukit Dugang (15/05/2002)	6.6	27.8	163	133.5	6.1	1.74	2	1.84	0.38	1.2	9.0	1.09	0.09	2.27	4.54	0.16
Kampung Jenderam (15/05/2002)	6.7	31.2	85	0.3	4.6	0.35	4	0.23	0.25	0.8	5.0	6.74	0.16	1.09	3.4	0.28
Bukit Changgang (15/05/2002)	6.3	32.4	104	85.3	5.1	1.21	1	1.23	0.00	1.2	18.0	5.54	0.6	3.49	4.39	0.22
Labohan Dagang (15/05/2002)	4.6	30.3	263	734.7	4.7	0.43	7	2.41	0.02	1.5	63.0	3.79	0.01	0.15	1.79	0.43

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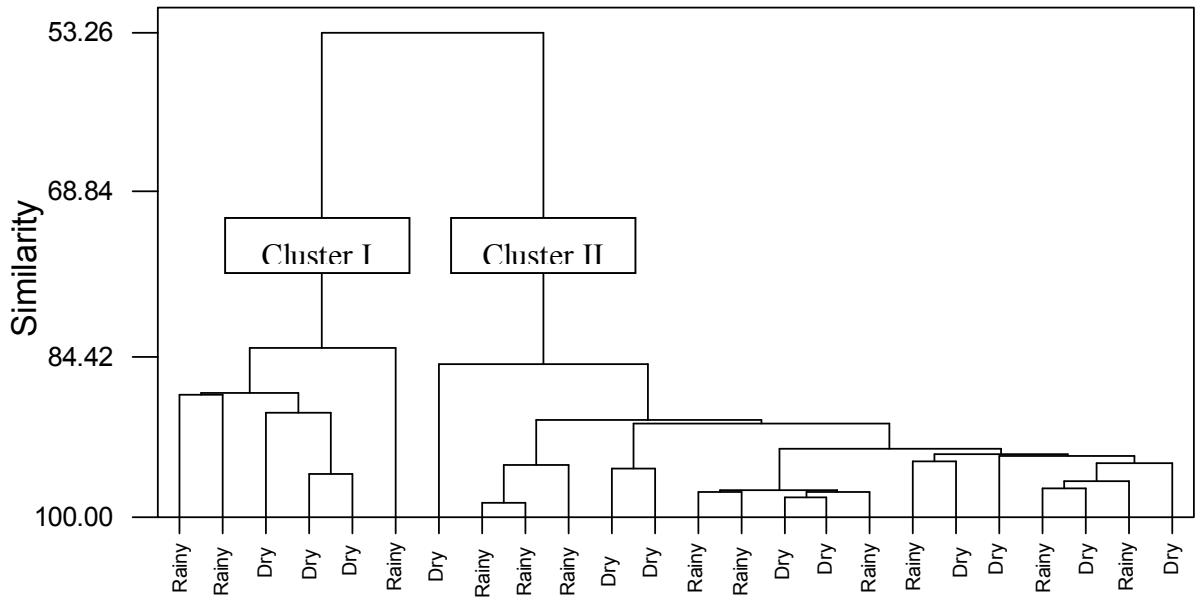

Figure 1. Seasonal dendogram calculated by the Ward method for the variables of Table 2 – four sampling plots with six sampling periods

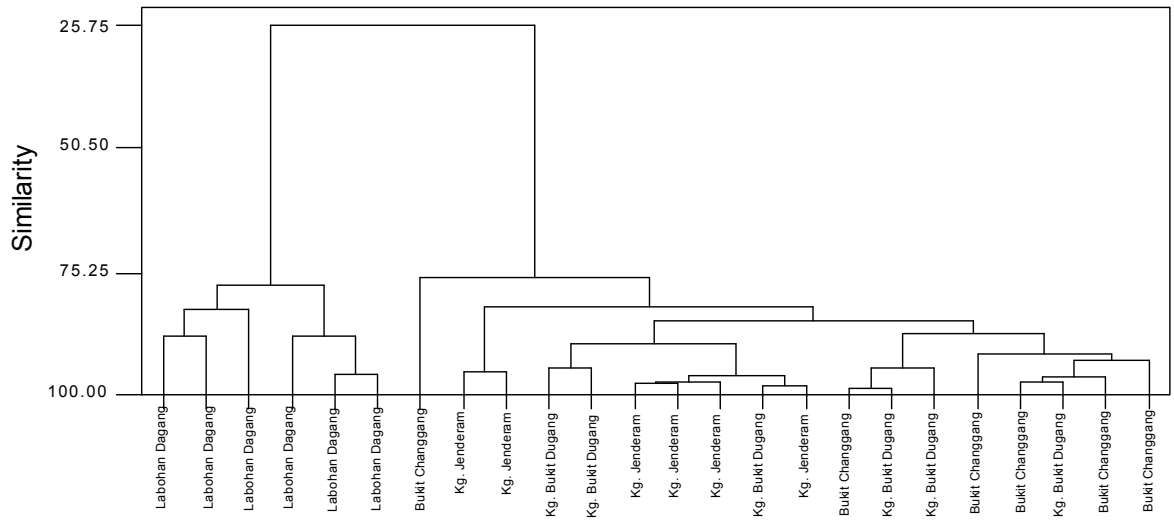

Figure 2. Sampling plot dendogram clearly separating Labohan Dagang and the other plots (Kg. = Kampung)

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The two groups of samples from plot 4 (Group 1) and plots 1, 2 and 3 (Group 2) join at a lower level of similarity in the sampling plot dendogram (Figure 2) compared to the seasonal dendogram (Figure 1). This demonstrates that from a hierarchical point of view the difference between the two separated groups (1 and 2) is larger in the sampling plot dendogram (Figure 2) compared to the seasonal dendogram (Figure 1). This is an indication that separation of sampling plots should be used as a significant factor in forming the basis for choosing sampling sites in order to study the effects of palm oil plantation on water quality. Searching for seasonal dependency based on the conventionally chosen sampling sites is consequently an ineffective exercise which involves high cost and much sampling time being wasted.

Principal component analysis

Table 4 shows the variance explained by the principal components obtained in a PCA. It clearly shows that most of the data variance is explained in the first 2 PCs (99.46%). This result is in agreement with the observed highly redundant information caused by the presence of several variables with high covariance.

Table 4. Variances of PCA for the first six PCs

PC	Variance (%)	Total
1	92.7	92.7
2	6.76	99.46
3	0.26	99.72
4	0.17	99.88
5	0.07	99.96
6	0.04	99.99

Figure 3 shows the scores of the objects (sampling sites) in a space spanned by PC1 and PC2, and the loadings of each feature (water quality variables) are shown for PC1 in Figure 4. In Figure 3, the score plot clearly shows two linearly separable clusters. The cluster on the right is formed by sampling sites in the Labohan Dagang plot while the rest of the sampling stations in the 3 sampling plots (Kampung Bukit Dugang, Kampung Jenderam and Bukit Changgang) form the other cluster. This further confirms, via visual inspection, the dendograms obtained from the hierarchical analysis results. Based on the PC1 loading diagram (Figure 4), it is quite clear that the difference between the two groups of sampling plots (Groups 1 and 2) is mainly due to the total suspended solid (TSS) (variable 4). Suspended solid is related to the natural erosion from the forest and agricultural area [33]. The second important variable is the conductivity (variable 3), which is due to the concentration of inorganic compounds in the water sample.

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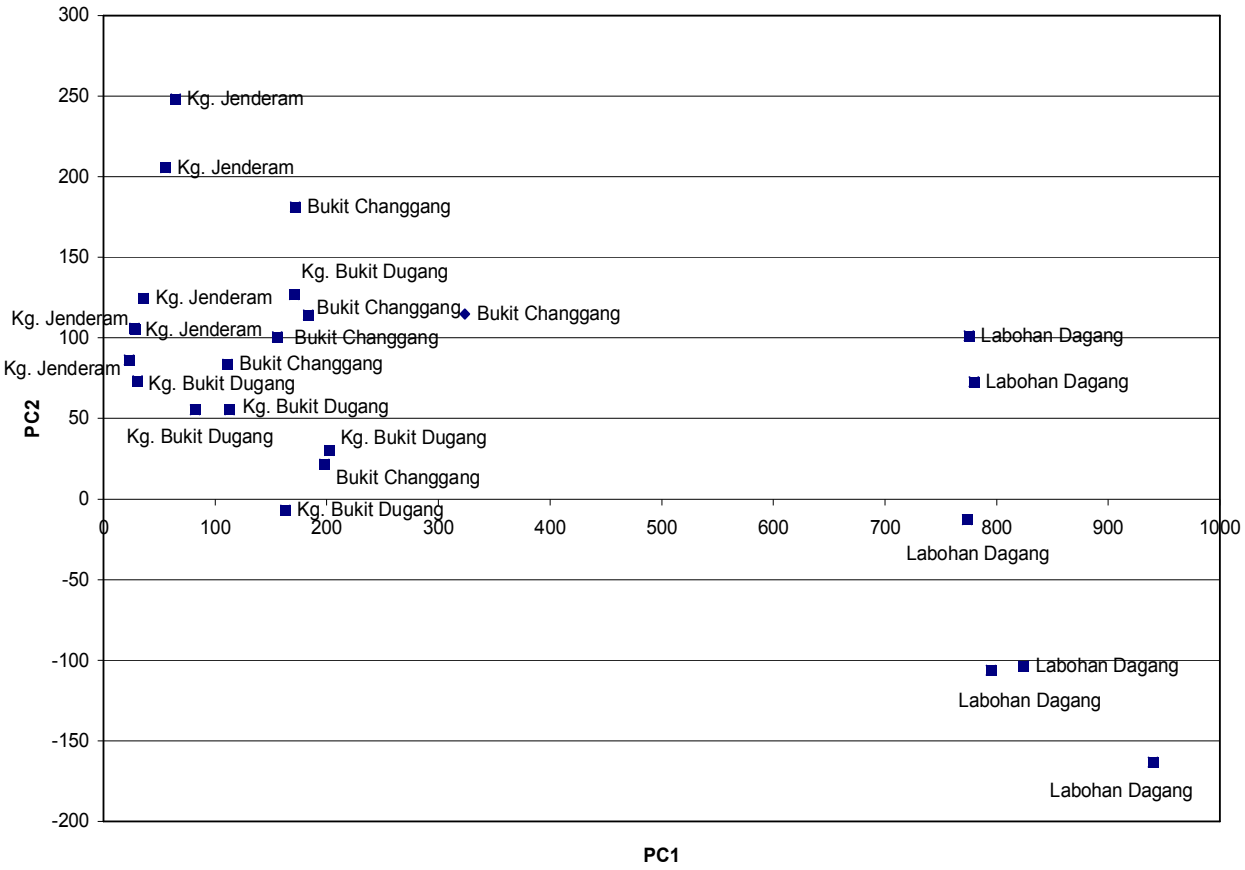

Figure 3. Principal component analysis (PCA) for four sampling plots (with six sampling periods)

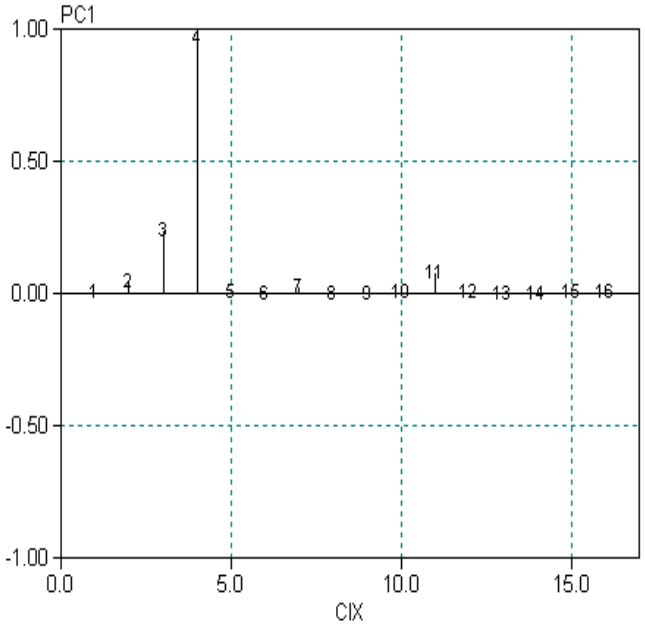

Figure 4. Plot of PC1 loadings

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Based on these results, it would be rather inappropriate to maintain the existing sampling sites, which were chosen based on economic reasons, if we are interested to study why certain areas exhibit high TSS. Sampling sites within group 2, for example, are rather redundant in this case. For further studies concerning the phenomena of high TSS, sampling sites within the plot of Labohan Dagang should be increased.

Design of experiments: Factorial design

If we are interested to study the interaction between two factors, such as the seasonal and sampling site factors as discussed in this work, we can use statistical methods classified under factorial design to do this. With the method, we can evaluate the effects of two or more factors simultaneously [34]. In this case, we try to interpret the results by testing whether there is an interaction effect between factor I (sampling plots) and factor II (weather condition). If the interaction effect between the factors is significant, one must be cautious in interpreting the phenomena. On the other hand, if the interaction effect is not significant, the focus of interpretation should be based on the potential differences between sampling plots (factor I) and weather condition (factor II).

Table 6 tabulates the ANOVA results obtained in testing for differences between two sampling plots (factor I): A (Labohan Dagang) and B (Kampung Jenderam, Bukit Changgang and Kampung Bukit Dugang). The decision rule in this test is to reject the null hypothesis if the calculated F value exceeds 5.32, which is the upper-tail critical value from the F distribution with 1 degree of freedom in the numerator and 18 degrees of freedom in the denominator. Because $F = 372.65 > F_u = 5.32$, and because the p-value = 5.38E-08 < 0.05, we reject the null hypothesis, and conclude that there is evidence of a difference between the two sampling plots in terms of the average amount of TSS. For sampling plot A, more TSS was observed (an average of 854.13 mg/L) compared to sampling plot B (an average of 138.67 mg/L).

In terms of factors in this study, if there was no interaction between sampling plots and weather condition factors, there should be little or no difference in terms of TSS between sampling plots A and B under both dry and rainy season. From Tables 5 and 6, it is observed that TSS in the dry season for station A was 655.47 mg/L above station B (735.30 vs. 79.83 mg/L). For overcast season, the average TSS for plot A was 715.46 mg/L above station B (854.13 vs. 138.67 mg/L). This difference is illustrated graphically by plotting the average values of each sampling plot for each weather condition. From Figure 5, we note that the difference between plots A and B is larger for overcast season than for dry season. However, this difference is relatively consistent for both dry and overcast season. This consistency in mean difference suggests that under different weather conditions (dry and overcast), the there is no change in TSS concentration. Pictorially, it is thus reasonable to conclude that there is indeed no relationship between sampling site and weather condition.

Identification of sources of pollution within the study area by PCA/factor analysis

Table 7 shows that among the six VFs, VF1 accounts for 18.4% of the total variance showing strong positive loadings on NO_3^- and SO_4^{2-}. Strong positive loading on NO_3^- is suspected to originate

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Table 5. Summary of TSS average and variance for plots A and B measured under two different weather conditions

Summary	**Overcast**	**Dry**	**Total**
A			
Count	3	3	6
Sum	2562.4	2205.9	4768.3
Average	854.1333	735.3	794.7167
Variance	7191.643	127.96	7164.25
B			
Count	3	3	6
Sum	416	239.5	655.5
Average	138.6667	79.83333	109.25
Variance	3853.243	3957.843	4162.843
Total			
Count	6	6	
Sum	2978.4	2445.4	
Average	496.4	407.5667	
Variance	157985.7	130525.3	

Table 6. ANOVA results in testing the difference in TSS measurements for sampling plots A and B

Source of Variation	**SS**	**df**	**MS**	**F**	**p-value**	**F crit**
Sample	1409594	1	1409594	372.6449	5.38E-08	5.317655
Columns	23674.08	1	23674.08	6.25856	0.036844	5.317655
Interaction	2700	1	2700	0.713781	0.422737	5.317655
Within	30261.38	8	3782.673			
Total	1466229	11				

from agricultural fields [11] where irrigated horticultural crops are grown and the use of inorganic fertilisers (usually as ammonium nitrate) is rather frequent. This practice could also explain the high levels of ammonia, but this pollutant may also originate from decomposition of nitrogen-containing organic compounds via degradation process of organic matters [35] such as proteins and urea occurring in municipal wastewater discharges. The presence of SO_4^{2-} may be attributed to the acid sulphate soils along the river banks.

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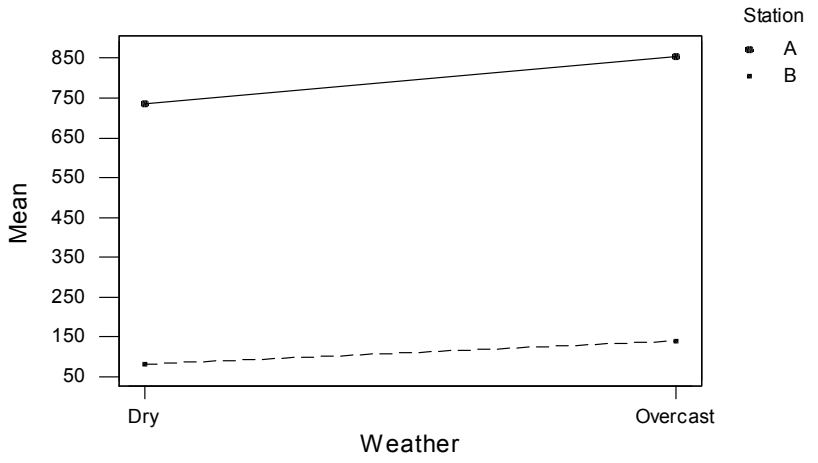

Figure 5. Interaction plot – data means for TSS

Table 7. Factor loading after varimax rotation

Variable	VF1	VF2	VF3	VF4	VF5	VF6
pH	0.065	-0.246	0.545	0.122	0.176	0.173
Temp.	0.286	-0.133	0.702	0.218	0.275	0.120
Cond.	0.333	**0.811**	-0.196	0.167	0.095	-0.050
TSS	0.676	0.129	0.106	-0.603	-0.054	-0.206
DO	-0.119	-0.172	**0.872**	0.025	-0.207	-0.033
BOD	0.019	-0.477	-0.063	0.163	**-0.813**	0.077
COD	0.551	-0.303	-0.263	-0.285	-0.396	-0.062
AN	-0.340	**0.798**	-0.186	-0.148	-0.116	-0.007
PO4	-0.198	0.102	0.014	0.079	0.014	**0.900**
NO3	**0.899**	-0.036	0.083	0.183	-0.284	0.001
SO4	**0.880**	0.043	-0.060	-0.123	-0.088	-0.191
Pb	-0.258	-0.287	0.010	-0.044	**0.809**	-0.025
Cd	-0.251	-0.134	-0.246	0.331	**0.762**	-0.060
Fe	-0.012	0.064	0.243	**0.861**	-0.027	0.040
Zn	-0.319	0.097	0.317	-0.466	0.556	-0.212
Cu	-0.026	-0.170	0.046	0.039	-0.162	**0.869**
Variance (%)	18.386	11.840	12.133	10.686	16.576	**10.947**
Cumulative (%)	18.386	30.227	42.359	53.046	69.621	**80.568**

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VF2, VF3 and VF4 account for 11.8%, 12.1% and 10.7% of the total variance and show strong positive loadings on AN, conductivity, DO and Fe content. The presence of AN is related to the influence of domestic waste and agricultural runoff [36-38] in their study, found that higher nitrogen levels were detected in agricultural waters, where fertilisers, manure and pesticides had been applied. Strong positive loadings on conductivity and DO could be explained by considering the chemical components of various anthropogenic activities which constitute point source pollution especially from industrial, domestic, commercial and agricultural runoff areas located at Hulu Langat, Cheras and Kajang districts. The presence of Fe basically represents the metal group originating from industrial effluents.

VF5 accounts for 16.6% of the total variance and shows strong positive loading on Pb and Cd and strong negative loading on BOD. Factories along the river bank may have contributed to the presence of Pb and Cd. VF6 accounts for 11% of the total variance and shows strong positive loading on PO_4^{3-} and Cu. The presence of PO_4^{3-} is most probably due to agricultural runoff such as livestock waste and fertilisers [39], industrial effluents, municipal sewage and existing sewage treatment plants because PO_4^{3-} is an important component of detergents [11].

Conclusions

This study has demonstrated that simple chemometric treatments are able to draw out from raw historical data information that would enable us to more effectively determine the "right" sampling sites for a particular objective, in order to reduce cost and time. In the case of the data obtained from the study, in order to determine the effects of palm oil plantation on water quality in the future, the researcher can determine the sampling sites in a more effective manner, relating the objective of the study to the type of sites to be chosen for sampling purpose.

Based on the original sampling sites, which were determined by economic reasons, it was found that the seasonal factor does not form a good basis of separation. Sampling sites and plots do not form reasonable clusters when weather condition is used as the factor. Thus, for the purpose of studying how seasonal change affects the water quality of this stretch of the basin, retaining the original sampling sites would prove ineffective. The sampling sites chosen in plots 1, 2 and 3 prove to be redundant for this purpose and should be reassessed. On the other hand, the separation of sampling plots due to suspended solid and conductivity, if these were historically available for the studied area, should motivate one to further study this phenomena. In designing sampling strategy for this purpose, TSS and conductivity must be considered as significant factors for reassessment to avoid redundant and unsuitable sampling sites.

This is just one simple example of the use of historical data and chemometric methods in determining new directions of sampling strategy, which results in the saving of sampling time and cost. Annual or even monthly reassessment of sampling sites based on this strategy may prove to be highly cost and time effective as well as direct research into new areas of study. The application of cluster

analysis, followed by principal component analysis as a classification method as demonstrated in this study, would help tremendously in future river pollution monitoring program.

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Full Paper

Screening and characterisation of bacteriocin-producing bacteria capable of inhibiting the growth of bovine mastitis

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Abstract: A total of 302 bacterial strains were isolated from 43 raw milk samples collected from dairy farms in Chiang Mai province. Out of these strains isolated, three strains were found to produce bacteriocins against *Streptococcus dysgalactiae* DMST10953, a bovine mastitis pathogen. These were *Lactobacillus fermentum* RMM701, *Streptococcus bovis* RMM703 and *Streptococcus bovis* RMM902, which exhibited bacteriocin activity at 40, 40 and 20 AU/ml respectively. Bacteriocins produced by these three strains were heat-stable up to 80 ^{o}C. Bacteriocins produced by *L. fermentum* RMM701 and *S. bovis* RMM902 were stable at pH 2.0 - 7.0 while that from *S. bovis* RMM703 was stable at pH 2.0 - 6.0. These bacteriocins were also found to be inactivated by proteolytic enzymes such as proteinase K and subtilisin A.

Keywords: bacteriocin, bovine mastitis, *Streptococcus dysgalactiae*

Introduction

Bovine mastitis is an inflammation of the mammary glands usually due to a microbial infection that affects milk production and quality, being one of the most significant causes of economic loss to the dairy industry [1]. *Staphylococcus aureus, Streptococcus dysgalactiae* and *Streptococcus uberis* are still the dominant pathogens [2]. Bovine mastitis is usually treated or prevented with intramammary

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antibiotics [3,4]. Although the use of antibiotics to control mastitis is normally very effective, it has some disadvantages, including the appearance of residues in the milk of treated cows [5] and the perceived connection to the emergence of antibiotic-resistant human pathogens, particularly the increased incidence of organisms such as methicillin-resistant *St. aureus*, which is prevalent in nosocomial infections in humans [6]. Thus the identification of alternative methods for controlling this illness is essential. One of these methods could be the use of bacteriocin.

Bacteriocins are proteinaceous antimicrobial agents and inhibit or kill closely related species of bacteria [7]. Some bacteriocins inhibit food spoilage and pathogenic microorganisms. They are therefore potentially useful as natural replacements for synthetic food preservatives or used in such medical and veterinary applications as the prevention and treatment of some infectious diseases [8,9].

In a previous study, nisin produced by *Lactococcus lactis* is effective against a wide range of Gram-positive bacteria, including mastitic pathogens such as *St. aureus* ATCC 2970, *S. dysgalactiae* ATCC 27957, and *S. uberis* ATCC 27958 [10]. In addition, when teat seal was blended with lacticin 3147 produced by *Lc. lactis* and infused into teats of nonlactating cows, the formulation reduced the incidence of clinical mastitis in teats that had been inoculated with *S. dysgalactiae* from 42% in control quarter to 6% in treated quarters [11].

The aims of this study are to screen bacteriocin-producing bacteria capable of inhibiting the growth of bovine mastitis pathogens and to study the physicochemical characteristics of these bacteriocins.

Materials and Methods

Bacterial strains and culture conditions

Three hundred and two bacterial strains were isolated from 43 raw milk samples in Chiang Mai province and used in the screening for bacteriocin production. *St. aureus* RMB203 and *St. aureus* RMB1601, were isolated from bovine mastitis. *S. dysgalactiae* DMST 10953 and *S. agalactiae* DMST 11366 were used as indicators. All lactic acid bacteria were cultivated in De Man, Rogosa and Sharpe (MRS) medium and incubated anaerobically at 37 ^{o}C for 16 h in anaerobic jars having a H_2+CO_2 environment generated with a BBL GasPak (Becton Dickinson Microbiology systems). Other non-lactic acid bacteria were grown in Brain Heart Infusion (BHI) medium at 37 ^{o}C for 16 h under aerobic condition. The bacteria were stored at -80 ^{o}C in 20% glycerol until needed.

Initial screening of bacteriocin-producing bacteria

The bacteriocin-producing bacteria were initially screened by spot agar test [12]. A total of 302 bacterial strains were grown in their appropriate culture medium and conditions: lactic acid bacteria were cultivated in MRS medium and incubated anaerobically at 37 ^{o}C for 16 h, and other non-lactic acid bacteria were grown in BHI medium at 37 ^{o}C for 16 h under aerobic condition. These bacteria was spotted onto BHI agar plate. After 16 h of incubation at 37 ^{o}C in aerobic condition, the plates were

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overlayed with soft BHI agar (0.7 % agar) containing indicator cultures. The plates were incubated overnight at 37 ^{o}C to assess the activity of these bacteria against each indicator strain.

Preparation of bacteriocin samples

Bacterial strains were cultivated in their appropriate culture medium and conditions. Extraction of bacteriocin was carried out using the method of Schillinger and Lucke [12]. Cells were removed by centrifugation at 5,000 rpm for 10 minutes at 4 ^{o}C. The supernatant were adjusted to pH 6.5 with 5M NaOH and 5M HCl to exclude the antimicrobial effect of organic acids. Inhibitory activity from hydrogen peroxide was eliminated by the addition of 1 mg/ml catalase, followed by filtration of the supernatant through a 0.2 µm pore-size nylon syringe filter. Supernatants were stored at -20 ^{o}C.

Bacteriocin bioassay

Antimicrobial activity against indicator organisms was determined using a well diffusion assay [12]. Pre-poured BHI agar plates were overlaid with BHI soft agar containing indicator cultures. Wells of 6 mm in diameter were cut into the agar plate with a cork borer and 50 µl of the cultured supernatant fluid was placed into each well. The plates were incubated overnight at 37 ^{o}C. The antimicrobial activity of the bacteriocin is defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn and is expressed in activity units per ml (AU/ml).

Identification of bacterial strains

Biochemical characteristics: Bacterial strains were cultivated in MRS medium and incubated anaerobically at 37 ^{o}C for 16 h. Sugar fermentation reactions were recorded by using the API 50 CH and API 20 STREP (BioMérieux).

Extraction, amplification and sequencing of bacterial DNA: DNA extraction was conducted by using the commercial Isoplant DNA extraction kit (NO. 314-02731, Nippon Gene, Japan) with the manufacturer's protocol. Extracted DNA was stored at -20 °C until needed. The 16S rDNA was amplified by polymerase chain reaction (PCR) using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 520R (5'-ACCGCGGCKGCTGGC-3') (Operon, Germany). PCR was performed in a PCR Sprint Thermal Cycler (Sprint, Thermal hybrid). For each reaction, a 50-μl reaction mixture was prepared. It consisted of 25 μl of Qiagen master mix, 2 μl of 27F primer, 2 μl of 520R primer, 20 μl of sterile H_2O, and 1 μl of 20 ng/ μl bacterial DNA. The amplification was programmed as follows: initial denaturation at 94 ^{o}C for 5 min, followed by 25 cycles at 94 ^{o}C for 1 min, 55 ^{o}C for 1 min, and 72 ^{o}C for 1 min. After these cycles, the reaction was maintained at 72 ^{o}C for 5 min and then cooled to 4 ^{o}C. Five microlitres of the PCR products were visualised after electrophoresis in a 1.5 % agarose gel and were subsequently visualised by UV illumination after ethidium bromide staining. The PCR products were purified by using the TaKaRa SUPREC^{TM}-PCR (Takara, Japan) according to the manufacturer's instructions, and analysis of all sequences was performed by First Base Laboratories Company (Malaysia).

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Sequence data analysis: All of the sequencing data were analysed with the BLAST (The National Centre for Biotechnology Information; NCBI).

Characterisation of bacteriocin

The bacteriocin samples were characterised with respect to heat, pH stability and susceptibility to denaturation by enzymes.

Heat resistance: Samples of bacteriocin were exposed to various heat treatments: 40, 60, 80, 100 and 121ºC. Aliquot volumes were then removed after 0, 30, 60 and 90 minutes [13] and assayed for bacteriocin activity.

pH sensitivity: Samples of bacteriocin were adjusted to pH 2, 4, 6, 8, 10, and 12 with 5N HCl or 5N NaOH. After incubating for 4 h at room temperature the bacteriocin samples were adjusted to pH 6.5 [13] and assayed for bacteriocin activity.

Enzyme treatment: The sensitivity of the bacteriocin to different enzymes was checked. The cell-free supernatant fluid at pH 6.5 was treated separately with protease and proteinase K at a final concentration of 1.0 mg/ml. Samples of bacteriocin were incubated with each enzyme for 2 h at 37 ºC. After that samples of bacteriocin were heated for 5 minutes at 100 ^{o}C to inactivate enzyme activity [14] and assayed for bacteriocin activity.

Results and Discussion

Isolation of bacteria and screening of bacteriocin producing bacteria

A total of 302 bacterial strains were isolated from 43 raw milk samples in Chiang Mai province. These isolates were tested for antimicrobial activity against 4 indicator strains. Only 148 strains showed inhibition zones when they were analysed by the spot agar test. Out of these strains, only three strains (RMM701, RMM703 and RMM902) produced bacteriocins against *S. dysgalactiae* DMST 10953, which is a bovine mastitis pathogen, when cell-free supernatants were analysed by the well-diffusion assay. Figure 1 shows the sizes of the inhibition zone against *S. dysgalactiae* when 10% (v/v) lactic acid was used as positive control. Bacteriocins produced by these three strains exhibited bacteriocin activity at 40, 40 and 20 AU/ml respectively (Table 1).

Table 1. Activity of bacteriocin produced by three bacterial strains against *Streptococcus dysgalactiae* DMST 10953

Strain	Inhibition zone (mm)*	Bacteriocin activity (AU/ml)
Positive control	12.4 $\pm$ 0.6	-
RMM701	9.3 $\pm$ 0.6	40
RMM703	9.7 $\pm$ 0.6	40
RMM902	9.7 $\pm$ 0.6	20

* well = 6.0 mm

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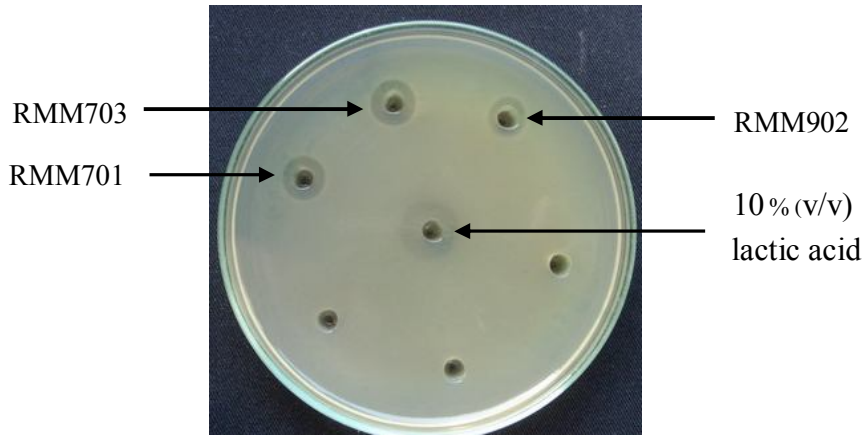

Figure 1. Results of the well-diffusion assay of three bacterial strains which produced bacteriocins against *Streptococcus dysgalactiae* DMST10953. (The rest of three wells showed negative results.)

Identification of bacterial strains

Strains RMM701, RMM703 and RMM902 were identified as *Lactobacillus fermentum*, *Streptococcus bovis* and *Streptococcus bovis* by 75.8, 97.6 and 97.6% homology respectively, based on API 50 CH and API 20 STREP (BioMérieux) profiles (not shown) and their physiological characteristics. The 16S rDNA was amplified from three bacterial strains. Fragments of about 500 bp were obtained (Figure 2.). The 16S rDNA sequence of RMM701, RMM703 and RMM902 were determined and compared with available 16S rDNA sequences in the Genbank database. The sequences were similar to *L. fermentum* RMM701, *S. bovis* RMM703 and *S. bovis* RMM902 by 99.1, 98.9 and 99.4 % homology respectively (Table 2). Thus, these strains were chosen for further characterisation.

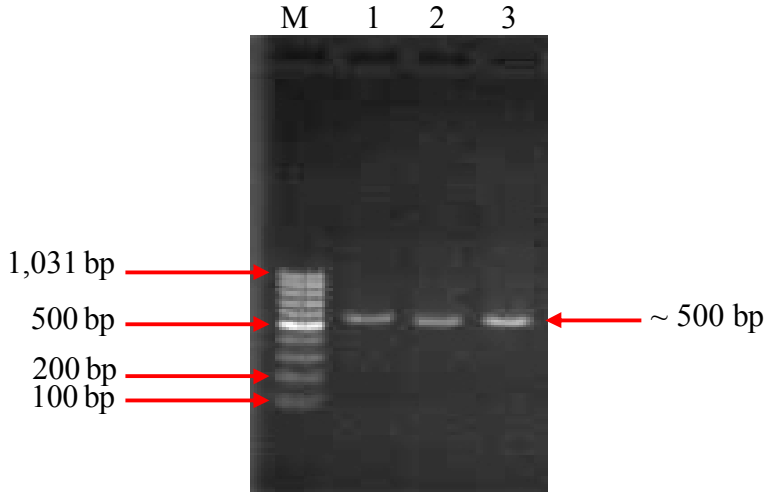

Figure 2. Ethidium bromide-stained agarose gel electrophoresis of amplified 16S rDNA of bacterial strains. Lane M: 100 bp DNA ladder used as molecular size marker; lane 1: RMM701; lane 2: RMM703; lane 3: RMM902.

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Table 2. Identification of bacteriocin-producing bacteria by 16S rDNA sequencing*

Bacterial strain	Bacteria	Accession number	Identity	% Homology
RMM701	*Lactobacillus fermentum*	DQ523484.1	522/527	99.1
RMM703	*Streptococcus bovis*	AB186306.1	525/531	98.9
RMM902	*Streptococcus bovis*	DQ467871.1	482/485	99.4

* Analysis date : 10 August 2007

Effects of temperature, pH and enzymes on the bacteriocin activity

The effects of heat, pH and enzymes on bacteriocin activity were determined using *S. dysgalactiae* DMST 10953 as indicator organism. Bacteriocins produced by the three strains (RMM701, RMM703 and RMM 902) were heat-stable at 80^{o}C for 60 min although their activities were reduced twofold. At 100 ^{o}C there was no detectable bacteriocin activity in these three strains (Figure 3). Heat sensitivity of these bacteriocins may be due to their non-complex, linear structures. Similar results of heat sensitivity were recorded for bozacin 14 [15]. Most bacteriocins were heat stable at 121 ^{o}C such as pediocin P [16], lactocin LC-09 [17] and nisin Z [18]. The heat stability may be due to the formation of small globular structures and the occurrence of strongly hydrophobic regions, stable cross-linkages, and a high glycine content [19].

Bacteriocins produced by *Leuconostoc* sp. RMM701 and *S. bovis* RMM902 were stable at pH 2-7, while that of *S. bovis* RMM703 was stable at pH 2-6 (Table 3). The loss of activity at higher pH could be due to change of conformation of the molecule. This result was similar to the properties reported for bacteriocins produced by other lactic acid bacteria such as Pediocin AcH [20], Pediocin A [21], Lactacin [22], Nisin [23] and bovicin HC5 [24].

These bacteriocins were inactivated by proteolytic enzymes such as proteinase K and protease (Table 4). Inactivation of antimicrobial activity by protease and proteinase K suggested that the substances could be antimicrobial peptides [16].

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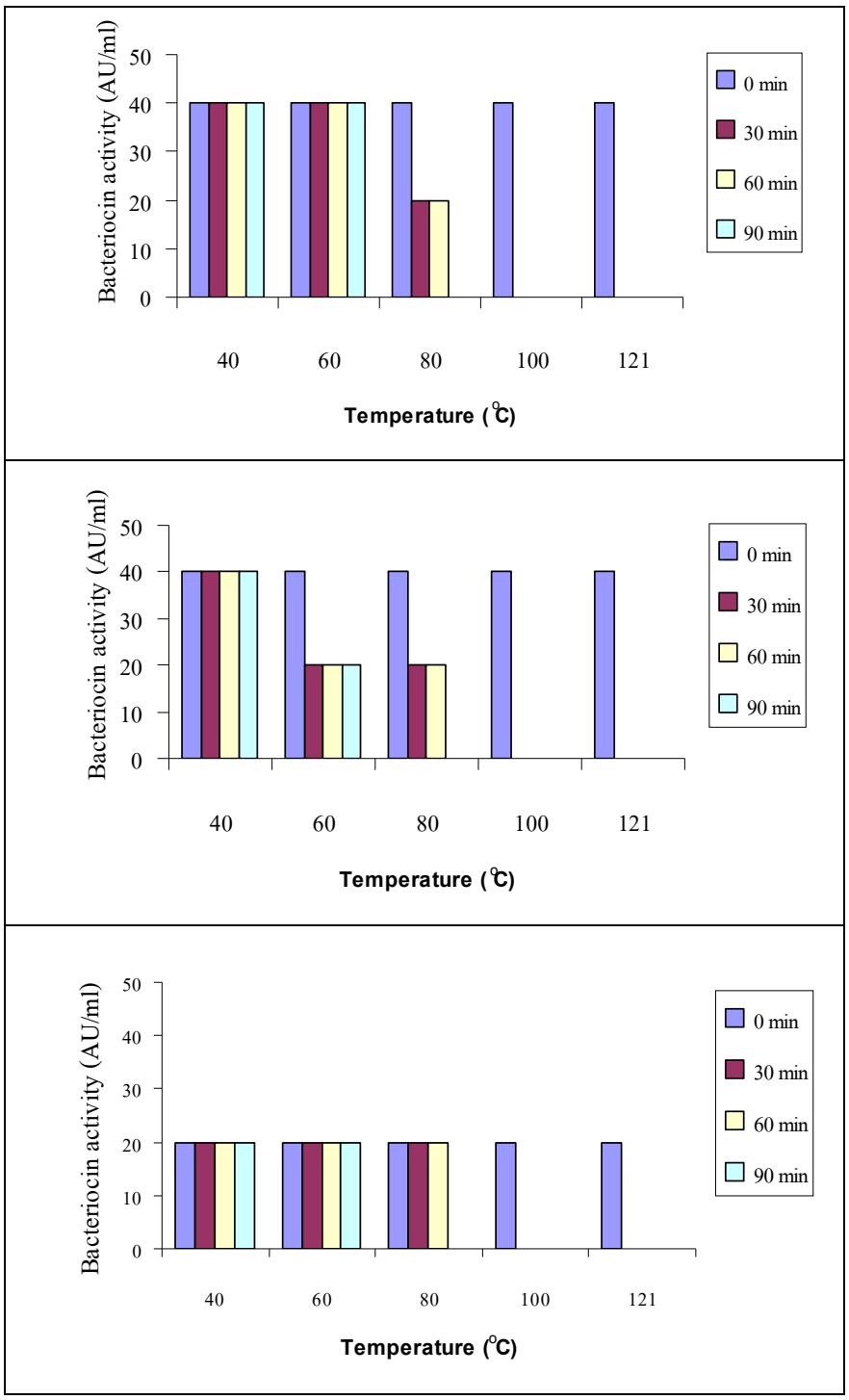

Figure 3. Effect of temperature on the activity of bacteriocin produced by each bacterial strain: (A) *L. fermentum* RMM701, (B) *S. bovis* RMM703, and (C) *S. bovis* RMM902

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Table 3. Effects of pH on activity of bacteriocin produced by each bacterial strain

pH	Bacteriocin activity (AU/ml)		
	L. fermentum RMM701	*S. bovis* RMM703	*S. bovis* RMM902
2	20	20	20
3	20	20	20
4	20	20	20
5	20	20	20
6	20	20	20
7	20	0	20
8	0	0	0
9	0	0	0
10	0	0	0

Table 4. Effects of enzyme on activity of bacteriocin produced by each bacterial strain

Enzyme	Bacteriocin activity (AU/ml)		
	L. fermentum RMM701	*S. bovis* RMM703	*S. bovis* RMM902
Control*	40	40	20
Proteinase K	0	0	0
Subtilisin A	0	0	0

* Crude extract of bacteriocins not treated with enzyme was used as control.

Conclusions

L. fermentum RMM701, *S. bovis* RMM703 and *S. bovis* RMM902 were isolated from raw milk samples from dairy farms in Chiang Mai province. They all produced bacteriocins against *Streptococcus dysgalactiae* DMST10953, a bovine mastitis pathogen. These bacteria exhibited bacteriocin activities at 40, 40 and 20 AU/ml respectively. Bacteriocins produced by these three strains were heat-stable up to 80 ^{o}C for 60 minutes, at which their activities were reduced twofold. Bacteriocins produced by *L. fermentum* RMM701 and *S. bovis* RMM902 were stable at pH 2.0 - 7.0 while that from *S. bovis* RMM703 was stable at pH 2.0 - 6.0. These bacteriocins were also found to be inactivated by proteolytic enzymes such as proteinase K and subtilisin A, suggesting that the substances could be antimicrobial peptides.

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Special Article

Thailand and brain drain

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Abstract: Brain drain has been the subject of research since the 1960s. This research has been hampered by a lack of accurate data from both source and receiving countries on migration and on the losses and gains to developing economies of skilled migration. However, despite these handicaps, research has been able to clearly show that trends are changing and the effect this is having is usually quite different for individual source countries.

Thailand, as a developing economy, could be regarded as a source country. Fortunately, Thailand has never ranked highly in terms of brain drain when compared to other states in Asia and while it may not be a significant problem it nonetheless needs to be monitored. Thailand is also somewhat unique in that the migration that has occurred has been almost equally split between secondary and tertiary educated Thais. Thailand also ranks low in terms of tertiary educated population who have migrated when compared to other countries in the region.

Globalisation is having a profound effect on the migration of skilled workers. As trade becomes increasingly free, barriers to the movement of services or people are also freed. As the better educated are encouraged to think globally, so too will they be inclined to move globally into the world community.

This paper examines Thailand's position with respect to brain drain, some of the lessons we have learned and some of the steps that are being taken to minimise the impact of the loss of skilled workers, with a particular focus on science and technology. The conclusion is that brain drain should not be viewed as an entirely negative development and that the positive outcomes should be recognised, encouraged and incorporated into policy.

Keywords: brain drain, brain gain, migration, manpower planning, science policy

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Background

Thailand is potentially vulnerable to the detrimental effects of brain drain as it cannot produce enough tertiary-educated researchers to fill the country's needs. Thailand fares poorly in this respect when compared to other economies in the region such as Korea, Taiwan and Singapore. Thailand has only 286 researchers engaged in research and development per million people (1990-2003), compared to 4,745 per million for Singapore and expenditure on R&D was only 0.2% of GDP in 2003 (compared to 2.2 for Singapore for the same year) [1]. In contrast to Taiwan and Korea [2,3], Thailand has invested considerable resources in tertiary education, thus making the 'cost' of brain drain proportionately higher.

However, balanced against this are other factors such as strong cultural and family roots, which tend to make migration a choice to be carefully considered. While there are often political and economic upheavals, few have been severe enough to warrant a mass outflow of skilled human capital.

Developed countries are often the providers of tertiary education, with developing countries making up one of their major markets. The recent trend has been for these developed countries to simplify, and in isolated cases encourage, migration from developing country students completing their education in the receiving country. Some 10% of America's S&T community is foreign [4]. In Australia it is as much as 25% [5]. In cases where education has been funded by the Thai government, a policy of bonding students has been in place since the 1950s. The standard is to require service amounting to twice the time spent overseas, or financial compensation amounting to two or three times the cost of the education. This is less unfair than it may at first appear when it is considered that recipients of government scholarships usually continue to receive their regular salaries whilst studying overseas. Unfortunately, there is no firm data to support the possibility that bonding restricts brain drain or the possibility that it could in fact have a negative effect, given the ever-changing scene with respect to worker mobility, both inside and outside of the country. Certainly it may be said that the bonding system causes some resentment amongst the bonded.

Many of the source countries where brain drain has been a major issue (e.g. Fiji, the Philippines, China, India and Korea) are often hardest hit in the health sector. This restricts their ability to provide basic services. Thailand is perhaps fortunate in this regard in that this sector has remained strong, from services to research. Health services are rapidly becoming a growth industry with the promotion of health tourism providing opportunities to help retain skilled resources in this area [6].

Trends

Recent research has helped to better define the trends in migration of skilled workers and three major categories have emerged. These are the basic brain drain, where there is almost a one-way outflow; reverse brain drain, where there is a trend for migrants to return to the source country; and circulating, where skilled human resources flow readily between the source and receiving countries, spending periods of time and employment in both [4,7].

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The latter two categories could both be considered gains for the source country. As globalisation increases, the trend is likely to favour the circulating worker.

Thailand also differs from other countries in the region in that the migration that has occurred has been almost equally split between secondary and tertiary educated Thais [8]. With a 2000 figure of 1.6% of the total population who migrated and had a tertiary education, Thailand ranks quite low when compared with, say, 29.4% for Malaysia [9].

Total migration from Thailand to the United States (as an example) has not increased over the last decade and, if anything, has decreased slightly. Nor was there any noticeable upsurge due to the financial crisis of 1997 [9]. While there is no disturbing trend in Thailand at the moment, this could change quite rapidly if the factors contributing to skilled migration are not recognised and monitored. For example, there has been a noted upsurge in skilled migration from Fiji on each occasion a coup occurs [10]. Another factor is the quality of tertiary education at home. Thailand has been fortunate in this regard and the situation continues to improve. However, in the Philippines, by way of example, the tertiary education sector has been largely privatised and prospective students are thus faced with a choice of pay at home or pay overseas, often choosing the latter. Having paid for their education in the receiving country and because of the special status afforded to Filipinos by countries like the US, they are disinclined to return. Both China and Vietnam were heavily affected in the past due to political upheaval and the effects of war. While no reliable figures are available for Burma, it can be assumed that brain drain is a major problem for the economy and future development of that country.

Does brain drain have a negative impact on the development of science and technology in the source country? The evidence suggests that the impact is different for each country. For countries such as Fiji, where 89% of migrants have been ethnic Indian and 15% of total migrants were skilled, this is equivalent to 4.7% of total government revenue – a result that is clearly negative [6]. Britain has more skilled professionals departing than any other country in the world. Yet Britain retains one of the most productive research sectors and is regarded as a leader in science [7]. This may be due to Britain producing a surplus, making competition for academic or research positions quite strong, thereby continually raising the quality standards.

Stark [4] argues that the potential for migration is in itself an incentive for workers to improve their skills and thus the marketability of their human capital. In effect, attaining a higher degree can open the door to joining the global community, should the domestic situation make this desirable.

Thailand also needs to be conscious of the fact that it is gradually becoming a receiving country and has the potential to enhance and take advantage of this. The Asian Institute of Technology is a good case in point. AIT has shown that it can attract skilled researchers from other countries, without necessarily using OECD-level salaries to achieve this. As opportunities for tertiary education in many neighbouring countries remain limited, Thailand can expect a greater influx of students from Burma, Laos, Cambodia and, to a lesser extent, Vietnam. If attractive employment opportunities are available, a number of these students could be expected to stay, adding to the skills pool in Thailand.

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Reversing the Trend

The single most critical factor for minimising the potentially deleterious effects of brain drain is creating the right domestic environment in the source country. The right environment is made up of a number of factors, apart from political and economic stability.

By creating the right environment it becomes possible to reverse brain drain and increase the number of circulating skilled workers, thus turning brain drain into a positive for the source country. However, it should be stressed that the first priority in creating the right conditions should be the domestic skilled workforce and special facilities should not be targeted specifically at attracting the return of migrants.

Factors that might make up the right environment are mostly within the government's ability to influence. In China and India, for example, the creation of science parks has had a major influence in encouraging the return of skilled workers [6,11]. Improvement of the quality of academic and research institutes has also been shown to be a major factor. Quality of the working environment is equally, if not more, important than issues such as salary. Thailand is working to create the right environment, including the recently opened Thailand Science Park and a number of technology-related industrial parks. Greater autonomy is being given to universities and research institutes. Universities are working closer with industry and the number of international programs is increasing. NSTDA has an on-going Reverse Brain Drain project which can hopefully be successful in strengthening the important overseas networks.

Innovative schemes that remove restriction are an important factor. These might include providing sabbatical leave or post-doctoral attachment at institutes in developed countries. Where possible, linkages between institutes should be encouraged where this might involve a migrant researcher. Joint research provides an opportunity for the migrant researcher to feel like they are participating in and contributing to their home country. The increase in international programs offered by universities in Thailand and the trend towards removing some of the government restrictions on universities are two positive trends making these institutions more likely to be attractive to overseas skilled Thais. Such researchers, whether they return or circulate, bring new knowledge and skills, language and a more entrepreneurial attitude towards science and technology to the source country [10].

Environment may also include cultural factors in the workplace and efforts should continue to eliminate gender bias, along with promotion based on seniority rather than skills or expertise. Admittedly, this will take time.

Thailand also has the opportunity to learn from the policies of other countries in the region. Taiwan in particular is an interesting case as it used to have a major problem with brain drain and has successfully managed to reverse this through far-sighted and patient policies. Apart from creating the right environmental factors, Taiwan has deliberately targeted its overseas skilled human resources by forming strong linkages with the receiving country's institutes or companies and providing the resources necessary to build on these linkages. With access to many major markets, Taiwan has been able to encourage direct investment from overseas companies employing Taiwanese nationals. Close

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collaboration between research institutes in Taiwan and the US, involving migrant researchers, has directly helped the advancement of science and technology in the source country [2,3].

Singapore is another interesting example in that it is primarily a receiving country and follows a policy of encouraging skilled migration from developed countries to complement her workforce. This is managed by targeting circulating foreigners, rather than permanent migration. Singapore has also had some success in attracting the return of top level Singaporeans, although this has been due to a combination of facilities and salary.

The lesson perhaps for Thailand here is that it is already regarded as an attractive destination for foreigners and this could be turned to advantage by making it conducive for skilled foreigners to seek employment and stay longer than normally they would. Thailand has also become a popular destination for retirees, many of whom are highly skilled and remain active. Some developing countries are even willing to provide salary top-up and other incentives for such overseas workers. To date, no concerted efforts have been made to identify these potential sources and tap their skills, and in fact harsher immigration rules and fees have become a major deterrent for this particular group. Thailand is also perhaps a special case in that an undocumented number of female researchers studying overseas tend to marry nationals of the receiving country. In the majority of cases, the husband would have at least equivalent skills. Greater effort could be made to turn such inevitable developments into a positive by creating the right environment for both husband and wife to return and work in Thailand.

One other aspect that is more difficult to quantify is the possibility that overseas Thais, because of the nature of their position or their work, may in fact be more useful in furthering Thailand's interests by remaining overseas. Trade access is probably the most obvious example. In the research area, there are likely to be cases where the Thai researcher has access to facilities for his/her research that could never be found in Thailand. This raises the question of what is the point in encouraging them to return if there are no facilities to pursue their interests? Would it not be better to wait until they are more senior, have greater experience and could adapt to a more broadly defined role in Thailand? Undoubtedly there are also cases where Thais are pursuing research overseas that is simply not a priority or for which there are limited employment opportunities in Thailand. Space research, nuclear physics and aircraft design are but some examples. This suggests that firstly, we should not be pursuing a policy of return at all costs and each case needs to be carefully assessed on its merits, and secondly, by directing effort into the networking approach the opportunity arises to make the most of advantages for Thailand of a returnee or an overseas Thai who remains in place. A long-term view is essential as circumstances for individuals change.

Countering Resentment

China in particular, and Vietnam to a lesser extent, have encountered problems with resentment from their domestic workers towards returning workers [6]. Many of these problems stem from the creation of special conditions or advantages for returning skilled workers who, in China, are referred to as "sea turtles" by the domestic "land tortoises." One lesson from this is that careful

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consideration needs to be given before creating any two-tiered system for domestic and returning workers. In Vietnam it is more likely due to the obvious affluence of many returning Vietnamese.

Thailand, however, is not immune to the problem of resentment. One factor is a real or imagined resentment of "superior" knowledge. Many researchers may have been involved in building their institutes from the ground up, having to struggle for every improvement and may feel resentful towards an outsider used to taking such improvements for granted. Such occurrences can be limited through skilful management. Some resentment can also be attributed to cultural factors. The returning worker, having been exposed for some time to a foreign culture and foreign working environment, may have trouble adjusting to the cultural norms. This is a major source of resentment. The domestic workforce wish to maintain the status quo at all costs. This constitutes their comfort zone where daily tasks are achieved by an unwritten set of rules. It can be difficult for a returning Thai, accustomed to the direct approach, to re-assimilate in such an environment. Faced with such difficulties, many returning Thais, and indeed foreign workers in Thailand, feel that their skills are under-utilised and under-appreciated. There have been some notable failures and the issue needs to be addressed. This is particularly important if the workplace culture in Thailand is to progress and develop more towards international standards.

Given the benefits that can accrue from the return of skilled Thais, prospective employers should carefully consider the possibility of resentment and attempt to minimise this.

Conclusions

For Thailand at least, brain drain is firstly not yet an issue of major concern and secondly, it should not be viewed in a negative light. Measures that might restrict the mobility of highly skilled workers are more likely to have negative effects. Skilled migration is inevitable and cannot be prevented by government. Time would be better spent on examining how to turn skilled migration to advantage. Some argument can be made that there are clear economic gains to be made from an increasingly mobile skilled workforce. Closer examination of the bonding system could be performed in the light of more recent research that has come to hand.

Thailand has been taking some positive steps to improve the environment for the highly skilled. Some facilities for researchers are now world-class. The next step will be a similar improvement in the private sector environment. This will ultimately enhance the possibility of either temporary or permanent returnees (brain gain). To counterbalance any negative impact that might occur from brain drain, Thailand should make better use of its natural assets to attract skilled researchers from developed nations. The first step would be an inventory of existing skilled foreign workers residing in Thailand and devising means to make use of these resources, if this is not already being done.

Thailand also needs to take stock of its overseas resources. A database of skilled Thais working abroad needs to be established and maintained. This would firstly enable a more accurate picture of the size and nature of brain drain. While this would be difficult for the private sector, researchers could be traced through their published work. This should then be followed by the establishment of networks, along the lines of the Taiwanese model, coupled with a flexible and

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innovative program to get this skilled resource involved in Thailand again. Such a move would also provide a baseline for Thailand to better monitor the effects of increasing globalisation and worker mobility.

Finally, managers in the public sector need to be conscious of the potential for resentment against returning skilled workers and serious efforts should be made to minimise this. The best way to achieve this would seem to be the reducing of any preferential treatment and attempting to not regard returnees as particularly special. However, simply casting returnees into a 'sink or swim' situation will not improve the chances of a longer stay and contribution. Each case requires sensitive handling and the key is creating a work situation that satisfies all parties. For Thailand, this is likely to be more successful than creating special environments just for returnees, as in the case of India.

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Full Paper

Effect of heat treatment on the antioxidant capacity of garlic

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Abstract: The determination of the antioxidant capacity of dry and wet-heated garlic, at 70, 100, and 121 °C by 3 different methods, namely ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolourization assay, and DPPH free radical scavenging activity, together with the determination of total phenolic content and formation of browning pigments of the same materials was carried out. The result showed that the antioxidant capacity of heated garlic was decreased by the decomposition of some phenolic and sulfur-containing compounds. However, when browning pigments developed, the antioxidant capacity of the heated brown garlic increased with the degree of browning, provided that it was not too dark. In addition, this study showed that ABTS and FRAP assay were better methods for expressing the antioxidant capacity of garlic due to its total phenolic content, although FRAP and DPPH assay were better if the antioxidant capacity of garlic was mainly caused by browning pigments.

Key words: garlic, antioxidant capacity, heat treatment

Introduction

Garlic (*Allium sativum* Linn.) is widely used as a food ingredient and a medicinal plant in many countries, especially in Asia. It is also a recommended Thai medicinal plant for the primary health care system [1]. Epidemiologic studies show an inverse correlation between garlic consumption and progression of cardiovascular disease. Garlic has been shown to inhibit enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation of oxidised erythrocytes and LDL,

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increase antioxidant status, and inhibit angiotension-converting enzyme in the body [2-3]. It also prevents DNA damage in essential hypertension [4].

Evidence from several investigations suggests that the biological and medicinal functions, such as antimicrobial, hypolipidemic, antioxidant, and antithrombotic properties that have been attributed to garlic are related to a variety of sulphur-containing compounds, including volatiles such as allicin, non-volatile water-soluble sulphur compounds such as S-allyl cysteine, and lipid-soluble sulphur compounds such as diallyl sulphide and diallyl disulphide [5-9]. Allicin is formed when alliin, a sulphur-containing amino acid, comes into contact with the enzyme alliinase when raw garlic is chopped, crushed, or chewed. Allicin scavenges hydroxyl radicals ($OH^{\bullet}$), and prevents the lipid peroxidation of liver homogenate in a concentration-dependent manner [10]. The antioxidant activity in the liposome system of diallyl sulphide, diallyl disulphide, S-ethyl cysteine, and N-acetyl cysteine derived from garlic was demonstrated, but this activity was lost when the temperature reached 65 °C [11].

Fresh, dried and fried garlic are used as food ingredients or seasonings in Thai cuisine. During the cooking or heating process, non-enzymatic browning reactions including Maillard reaction, caramelisation, and chemical oxidation of phenols occur. The antioxidant activity of the products from Maillard reaction [12-14] and caramelisation [15] have been reported. On the other hand, the decrease in antioxidant capacity of boiled garlic at 100 °C has been found [16-17].

In this present work we report the determination of the antioxidant capacity of garlic during the drying and wet heating process at 70, 100, and 121 °C, which are representatives of three heating conditions, i.e. heating below boiling point of water or pasteurisation, heating at boiling point of water or sterilisation of high acid and acid food (pH < 4.5), and heating above boiling point of water or sterilisation of low acid food (pH > 4.5). The determination was done by three different methods, viz. ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolourization assay, and DPPH free radical scavenging assay. Total phenolic content and formation of browning pigments (absorbance at 420 nm) were also determined. The correlations of antioxidant capacity (%) with total phenolic content (%), and of antioxidant capacity (%) with absorbance at 420 nm were then analysed.

Materials and Methods

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Aldrich. TPTZ (2,4,6-tripyridyl-s-triazine) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma. ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)], Folin-Ciocalteau phenol reagent, ferric chloride, ferrous sulphate, gallic acid, glacial acetic acid, hydrochloric acid, sodium acetate, potassium persulphate, sodium carbonate, and vitamin C were purchased from Fluka. All chemicals were of analytical grade.

Sample preparation

Fresh garlic was purchased from a local market and edible portion was homogenised with a blender. Sample tubes (25 x 150 cm) containing 2 g each of blended garlic were dried in a hot air oven at 70, 100, or 121 °C. For wet heating, 10 ml of deionised water was added to the blended garlic in each

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sample tube before heating in a water bath at 70 or 100 °C, or in an autoclave at 121 °C. A sample tube was collected 10 times during a maximum of 10 hours of heating period. Sample extraction method of Leong and Shui [18] was modified. Ten ml of deionised water were added to the collected dried sample tube. (For wet heating, deionised water had been added before heating.) The extraction was done by vortex mixing for 1 min. The mixture was then filtered through a Whatman filter paper no. 1. The filtrate was adjusted to 10 ml by deionised water and this extract was used for all assays. An extract of blended fresh garlic was prepared for comparison and the weight change during the drying process also was recorded.

Ferric reducing antioxidant power (FRAP) assay

FRAP, a method for measuring total reducing power of electron-donating substances, was applied according to Benzie and Strain [19]. Briefly, 6 ml of working FRAP reagent (0.1 M acetate buffer : 0.02 M $FeCl_3$: 0.01 M TPTZ = 10 : 1 : 1) prepared daily were mixed with 20 µl of extract sample. The absorbance at 593 nm was recorded after a 30-min incubation at 37 °C. FRAP values were obtained by comparing with standard curves created by Fe^{2+} (0 - 14 µg), Trolox (0 - 35 µg) and vitamin C (0 - 15 µg), and reported as mg Fe^{2+}, Trolox and vitamin C equivalent per gram of sample (dry weight).

ABTS radical cation decolourization assay

The method of Re et al. [20], based on the ability of antioxidant molecules to quench the long-lived ABTS radical cation ($ABTS^{\bullet+}$), was modified. $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 - 16 hours before use. The $ABTS^{\bullet+}$ solution was diluted with deionised water and 95 % ethanol (1 : 1) to an absorbance of 0.70 ($\pm$ 0.02) at 734 nm. Twenty microlitres of the extract were mixed with 6 ml of the diluted $ABTS^{\bullet+}$ solution. The decrease of absorbance was recorded at 1 min after mixing. Trolox (0 - 30 µg) and vitamin C (0 - 20 µg) were used as standards, and the results were reported as mg Trolox and vitamin C equivalent per gram of sample (dry weight).

DPPH free radical scavenging activity

The method of Brand-Williams et al. [21], based on the reduction of DPPH radical solution in the presence of hydrogen donating antioxidants, was used with some modification. DPPH radical solution (0.8 mM) in 95% ethanol was prepared. One thousand microlitres of the extract were diluted to 5.4 ml using deionised water and 95 % ethanol (1 : 1) before 0.6 ml DPPH radical solution was added and the mixture shaken vigorously. The decrease of absorbance was recorded at 1 min after mixing. Trolox (0 - 50 µg) and vitamin C (0 - 40 µg) were used as standards, and the results were reported as mg Trolox and vitamin C equivalent per gram of sample (dry weight).

Total phenolic content (TPC)

The Folin-Ciocalteau micro method of Waterhouse [22] was used. Sixty microlitres of the extract were diluted with deionised water to 4.8 ml, and 300 µl of Folin-Ciocalteau reagent were added and the mixture shaken. After 8 min, 900 µl of 20% sodium carbonate were added and mixed. The solution

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was left at 40 °C for 30 min before the absorbance at 765 nm was read. Gallic acid (0 - 50 μg) was used as standard, and the results were reported as mg gallic acid equivalent per gram of sample (dry weight).

Formation of browning pigments

Formation of browning pigments was determined by the official method [23]. The absorbance of extracts was measured at 420 nm.

Calculation and statistical analysis

The values of FRAP, ABTS, DPPH, and TPC (mg standard equivalent per gram of sample (dry weight)) were calculated using the equation below:

$$\text{Values of FRAP, ABTS, DPPH, and TPC (mg standard equivalent per gram of sample (dry weight))} = \frac{[(SA - BA) / (Slope)]\,[10 / U]}{[2]\,[1\text{-}MC][1{,}000]}$$

where: SA = Sample absorbance for FRAP value and TPC or absorbance decrease of sample for ABTS and DPPH values
BA = Blank (no extract) absorbance for FRAP value and TPC or absorbance decrease of blank for ABTS and DPPH values (extract was substituted by deionised water for blank)
Slope = Slope of standard curve
[10 / U] = Total volume of extract (10 ml) / Used volume of extract (ml)
[2] = Weight of used sample (g)
MC = % Moisture content / 100
[1,000] = Factor for changing μg to mg

Changes of antioxidant capacity, TPC, and weight (%) were calculated using the equation below:

$$\text{Changes of antioxidant capacity, TPC, and weight (\%)} = \frac{\text{Value of collected sample x 100}}{\text{Value of blended fresh garlic}}$$

Each experiment was performed in triplicate and was conducted on separate purchased samples (triple measurements for each purchased sample). The bivariate correlations between changes of antioxidant capacity and total phenolic content, and between changes of antioxidant capacity and absorbance at 420 nm were analysed.

Results and Discussion

In this experiment, water was used as extracting solvent since about 97% of garlic components are water-soluble [6], and some previous work also confirmed that water is a good solvent for measuring antioxidant capacity and TPC of garlic extract [16,24], although a better result with hexane has been obtained by Leelarungrayub et al. [25]. Antioxidant capacity and TPC of aqueous fresh garlic extract are shown in Table 1. Values of antioxidant capacity and TPC of aqueous garlic extract have been

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reported by several workers but they are difficult to compare because of the differences in sample sources, sample preparations, methodology details, standards used, among others. For example, Gorinstein et al. [16] reported ABTS = 26.1 ± 2.4 μmol Trolox equivalent per gram of sample (dry weight), and TPC = 11.42 ± 1.45 mg gallic acid equivalent per gram of sample (dry weight). Jastrzebski et al. [17] reported FRAP = 3,400 ± 270 mmol Trolox equivalent per 100 grams of sample (fresh weight), DPPH = 69 ± 5.1 % inhibition, and TPC = 49.3 ± 3.1 mg gallic acid equivalent per 100 grams of sample (fresh weight). Wangcharoen and Morasuk [24] reported FRAP, ABTS and DPPH = 0.14 ± 0.04, 1.06 ± 0.11 and 0.16 ± 0.03 mg vitamin C equivalent per gram of sample (fresh weight) respectively, and TPC = 0.41 ± 0.10 gallic acid equivalent per gram of sample (fresh weight). Leelarungrayub et al. [25] found ABTS = 1462 ± 22 mg extract equivalent to 1 mg of Trolox, and TPC ≈ 3,800 mg gallic acid equivalent per kg of dry weight of extract. Holvorsen et al. [26] found FRAP = 0.21 – 0.24 mmol $FeSO_4.7H_2O$ equivalent per 100 grams of fresh weight of edible portion, and Nuutila et al. [27] found DPPH = 62.1, 60.9, and 43 % inhibition, and TPC = 75 ± 8.8, 115 ± 12.9, and 95 ± 7.8 mg gallic acid equivalent per kg of freeze-dried samples of Finnish organic garlic, Finnish garlic, and Hungarian garlic, respectively.

Table 1. Antioxidant capacity by various methods and total phenolic content (TPC) of aqueous extract of fresh garlic samples (mg standard equivalent per gram of sample (dry weight))

Standard	FRAP	ABTS	DPPH	TPC
Fe^{2+}	0.56 ± 0.15			
Trolox	1.13 ± 0.31	5.17 ± 0.54	0.57 ± 0.08	
Vitamin C	0.44 ± 0.12	3.41 ± 0.35	0.48 ± 0.07	
Gallic acid				1.29 ± 0.19

Changes of antioxidant capacity (%), TPC (%), weight (%), and 420 nm absorbance of the aqueous extract of garlic samples during heating process are shown in Figures 1 and 2. In the case of drying (Figure 1), the antioxidant capacity values and TPC tended to decrease with drying time when garlic samples were dried at 70 °C, except the DPPH value, which seemed to increase at 10 hours. The weight of garlic samples rapidly decreased in the first three hours and was steady after 4 hours, whilst the absorbance at 420 nm evidently decreased in the first and the second hour and gradually increased after the third hour. At 100 and 121 °C, all antioxidant capacity values and TPC decreased and reached the lowest point at 2.5 and 1.5 hours respectively, before increasing when the drying time was further increased. The weight of garlic extracts rapidly decreased in the first hour and was constant after that

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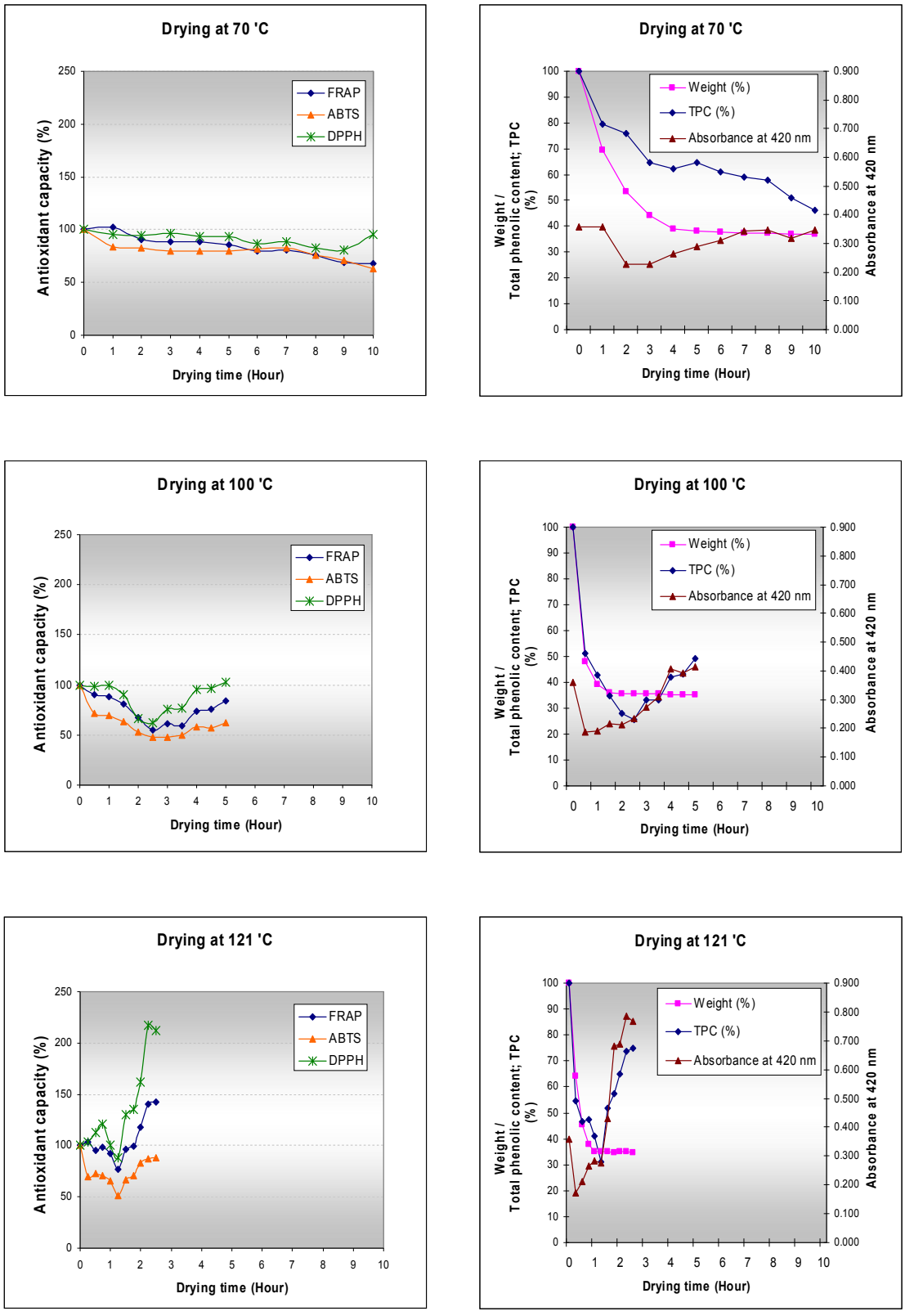

Figure 1. Antioxidant capacity, total phenolic content, and absorbance at 420 nm of aqueous extracts of garlic samples, together with weight of garlic samples during drying process at 70, 100, and 121 °C

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time, whilst the absorbance at 420 nm decreased to a minimum at about 0.5 hour before it evidently increased after that.

In the case of wet heating (Figure 2) at 70 °C, FRAP and ABTS values tended to decrease for 70 min before slightly increasing after that time, whilst DPPH value slowly increased until about 30 min, after which it slowly decreased and started to increase again after 70 min. At 100 °C, ABTS value steadily decreased, whilst FRAP and DPPH values seemed to increase for about 20 min before decreasing steadily for FRAP and with fluctuation for DPPH. This result agrees with that of Gorinstein et al. [16], who reported 13 – 17 % decrease of ABTS assay for boiled garlic at 100 °C for 20 min, and also agrees with that of Jastrzebski et al. [17], who showed a significant decrease of FRAP and DPPH assay of boiled garlic at 100 °C for 40 and 60 min. On the other hand, at 121 °C of wet heating, DPPH value increased rapidly, whilst FRAP and ABTS values also did albeit less so and only after an initial decrease.

The decrease of antioxidant capacity of garlic extract upon heating was most probably due to the decomposition of some phenolic compounds and also some sulphur-containing compounds such as diallyl sulphide, diallyl disulphide, S-ethyl cysteine, and N-acetyl cysteine, which would be lost when the temperature reached 65 °C [11]. However, when browning pigments were formed, the antioxidant capacity of the heated brown garlic was regained as a result of certain compounds, including phenolic compounds, being created during the browning reactions [12-15]. The browner garlic, with higher value of absorbance at 420 nm, expressed higher antioxidant capacity, but at the end of the heating period at 121 °C, the antioxidant capacity started to decrease (Figures 1 and 2). This shows that the antioxidant capacity of the heated brown garlic would be dropped if it was heated for a long time so that its colour was too dark.

From the results of bivariate correlation, the correlation coefficient (a number between -1 and 1 which measures the degree to which two variables are linearly related) of changes of antioxidant capacity (%) with TPC showed that ABTS values were higher correlated with TPC in all cases of drying process, followed by FRAP and DPPH values respectively. In the case of wet heating process, FRAP values seemed to be higher correlated with TPC than ABTS and DPPH values respectively. The bivariate correlation of changes of antioxidant capacity (%) with absorbance at 420 nm showed that FRAP and DPPH values were highly correlated with browning pigment formation in drying and wet heating process at 121 °C (Table 2). These differences might be due to the fact that ABTS and DPPH method involve free radicals reacting with phenolic and browning pigment compounds, while for FRAP assay, it is a method for measuring total reducing power of electron donating substances, which is not directly related to free radical reactions and not as specific as ABTS and DPPH assay.

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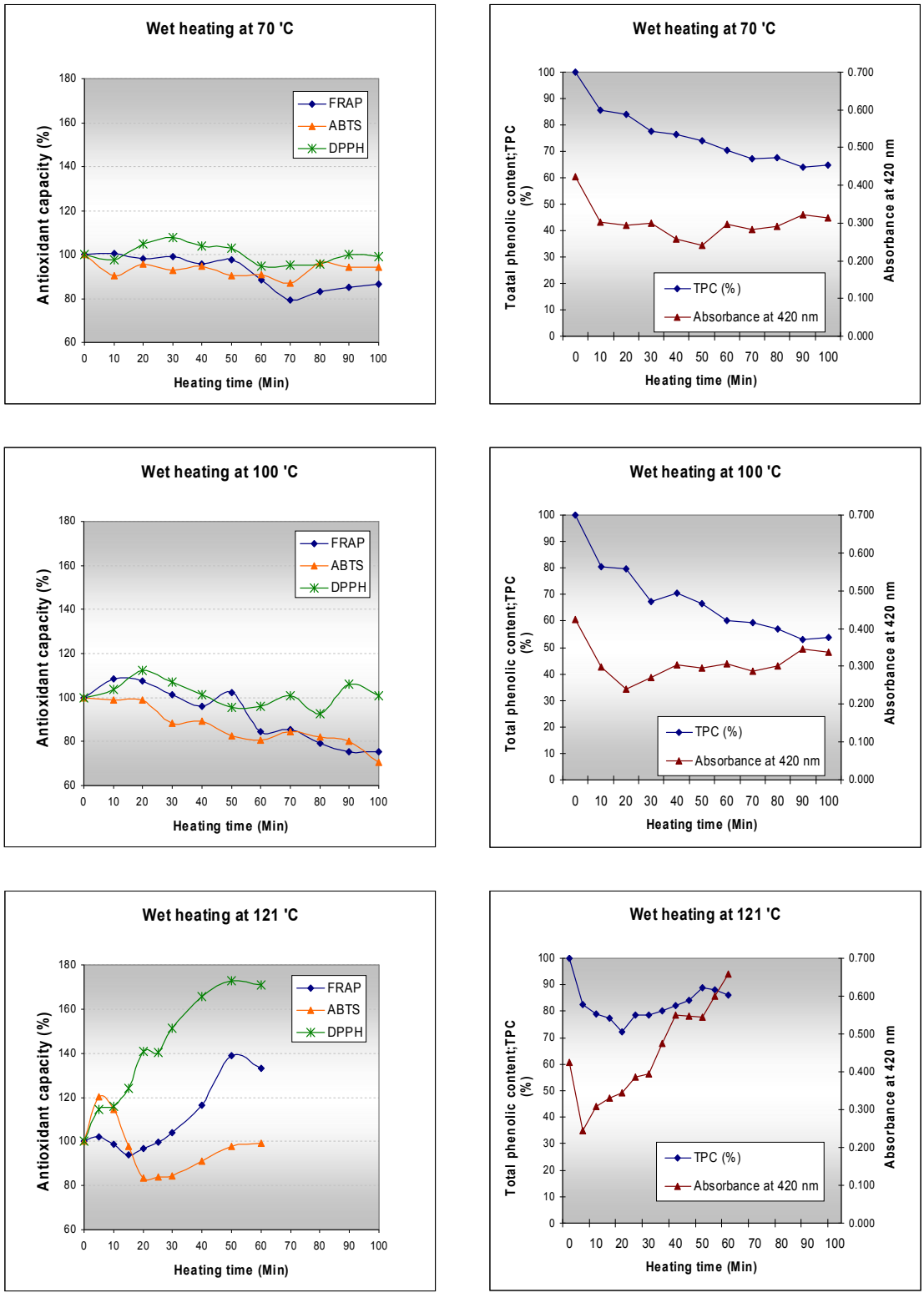

Figure 2. Antioxidant capacity, total phenolic content, and absorbance at 420 nm of aqueous extracts of garlic samples during wet heating process at 70, 100, and 121 °C

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Table 2. Correlation coefficients of changes of antioxidant capacity with total phenolic content (TPC) and with absorbance at 420 nm of aqueous extract of garlic samples during heating processes

Drying process		FRAP	ABTS	DPPH
70 °C	Total phenolic content (TPC)	0.891	0.919	0.636
100 °C		0.799	0.941	0.616
121 °C		0.597	0.961	0.436
70 °C	Absorbance at 420 nm	-0.114	0.050	-0.151
100 °C		0.090	0.100	0.377
121 °C		0.757	0.419	0.834
Wet heating process				
70 °C	Total phenolic content (TPC)	0.801	0.489	0.313
100 °C		0.768	0.893	0.243
121 °C		0.374	0.271	-0.153
70 °C	Absorbance at 420 nm	0.156	0.637	-0.131
100 °C		-0.275	-0.017	-0.315
121 °C		0.847	-0.306	0.774

Conclusions

This work has shown that the antioxidant capacity of garlic is decreased by both drying and wet heating. However, if browning pigments are developed from non-enzymatic browning reactions, the brown garlic will regain its antioxidant capacity, which may increase to an appreciably higher level than the starting value depending on the degree of browning. This expresses the high antioxidant capacity of heated brown garlic, which is normally used as an ingredient in many Thai food recipes.

Acknowledgement

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Full Paper

Isolation of acetic acid bacteria from honey

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Abstract: Four thermotolerant acetic acid bacteria designated as CMU1, CMU2, CMU3 and CMU4 were isolated from six honey samples produced by three native bee species in northern Thailand, namely the dwarf honey bee (*Apis florea*), Asian honey bee (*A. cerena*) and giant honey bee (*A. dorsata*). All isolates were tested for their tolerance to acetic acid and ethanol at 30°C and 37°C. It was found that they grew only in a medium containing 1% (v/v) acetic acid at 30°C. However, isolate CMU4 showed the highest toleration to ethanol, viz. 10% (v/v) and 9% (v/v) at 30°C and 37°C respectively. Morphological and biochemical examination indicated that all isolates were members of the genus *Gluconobacter*.

Keywords: acetic acid bacteria, honey, enrichment culture technique, *Gluconobacter*

Introduction

Honey is a nectar and sweet deposit from plants which is gathered, modified and stored in the honeycomb by honey bees [1]. There are five major bee species used in honey production industry in the northern part of Thailand. Four native species are the dwarf honey bee (*Apis florea*), small dwarf honey bee (*Apis adreniformis*), Asian honey bee (*A. cerena*) and giant honey bee (*A. dorsata*). The remaining European honey bee (*A. mellifera*) is the only introduced species [2].

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Acetic acid bacteria are a large group of obligate aerobic Gram-negative bacteria which are commonly found in association with various kinds of sugary material. They are divided into four groups, namely *Acetobacter*, *Acidomonas*, *Frateuria* and *Gluconobacter*, these being slightly different in some physiological characteristics [3]. The two most studied acetic acid bacteria are *Acetobacter* and *Gluconobacter* due to their economical importance. Acetic acid bacteria are found in association with honey and honey bees [4-6]. However, to date, apparently no studies attempting to isolate these bacteria from Thai honey have been reported. In this study, the selective enrichment procedure for the isolation of acetic acid bacteria of particular thermotolerant strains from honey was carried out.

Materials and Methods

Selective isolation of acetic acid bacteria by enrichment culture technique

A total of six honey samples from dwarf honey bees (*Apis florea*), Asian honey bees (*A. cerena*) and giant honey bees (*A. dorsata*) were collected from local honey farms in San Kamphang district, Chiang Mai, Thailand. Approximately 1 ml of each sample was added to the enrichment broth (20 ml) containing 0.5% glucose, 2% glycerol, 1% yeast extract, 1% peptone, 1.5% potato extract, 4% ethanol and 1% Acti-dione (an antifungal). The mixture was incubated at 37 ^{o}C for up to one week. After 1, 3 and 5 days of incubation, one loop-full of enrichment broth was streaked onto a potato agar plate (containing 0.5% glucose, 2% glycerol, 1% yeast extract, 1% peptone, 1.5% potato extract, 4% ethanol (v/v) and 0.003% bromcresol purple). All colonies showing a yellow halo zone were collected for further study. The change of pH in the enrichment broth was also observed after 1, 3, 5 and 7 days of incubation.

Phenotypic characterisation of isolates

Morphological, physiological and biochemical characteristics of all pure isolates were examined according to Bergey's Manual of Determinative Bacteriology [3]. The ability of the isolate to oxidise acetate to CO_2 and H_2O was used to distinguish between members of the genera *Acetobacter* and *Gluconobacter*.

Acid and alcohol tolerance of isolates

All isolates were tested for their ability to grow in seed culture agar supplemented with 1-4 % (v/v) acetic acid and that containing 5-15% (v/v) ethanol at 30 and 37 ^{o}C. Inoculum was prepared by growing each isolate on seed culture medium for 48 h and then transferred to the test plates. All plates were incubated for up to 5 days. The growth of each isolate was compared between the two temperatures.

Results and Discussion

Four isolates of acetic acid bacteria (Table 1) were successfully recovered from honey samples using enrichment technique. Two isolates, CMU1 and CMU2 were isolated from honey of *A. florae* whereas isolate CMU3 and CMU4 were from honey of *A. cerena* and *A. dorsata* respectively. The

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successful isolation was due to the efficacy of enrichment culture to promote the growth of acetic acid bacteria present in the samples. It is evident from Figure 1 that the numbers of acetic acid bacteria in the culture broth increased with time during the enrichment period. As a result, the pH values were decreased accordingly (Figure 1). Many reports have also addressed the usefulness of the enrichment culture technique in selective isolation of targeted microorganisms [7-10].

Table 1. Morphological characterisation of acetic acid bacterial isolates

Isolate	Source of honey	Days of enrichment	Gram stain and morphology
CMU1	*Apis florae*	3-7	Gram-negative, short rod
CMU2	*Apis florae*	3-7	Gram-variable, short rod
CMU3	*Apis cerena*	7	Gram-variable, short rod
CMU4	*Apis dorsata*	7	Gram-variable, short rod

As shown in Table 1, Gram staining revealed that the isolates were either Gram-negative or Gram-variable, short rod bacteria, which is a typical character of acetic acid bacteria. All of them were identified as *Gluconobacter* sp. due to their inability to oxidise acetate (Table 2). All isolates recorded the same phenotypic profile among themselves, thus suggesting that they belonged to the same taxa. Most acetic acid bacteria are known to be mesophilic with an optimum temperature for growth around 30°C. A slight increase in temperature results in a dramatic decrease in growth of these organisms [11,12]. However, all isolates obtained in this study grew well at 37°C, the character which suggested that these isolates may be thermotolerant strains. No members of the genus *Acetobacter* were obtained from any of the samples tested.

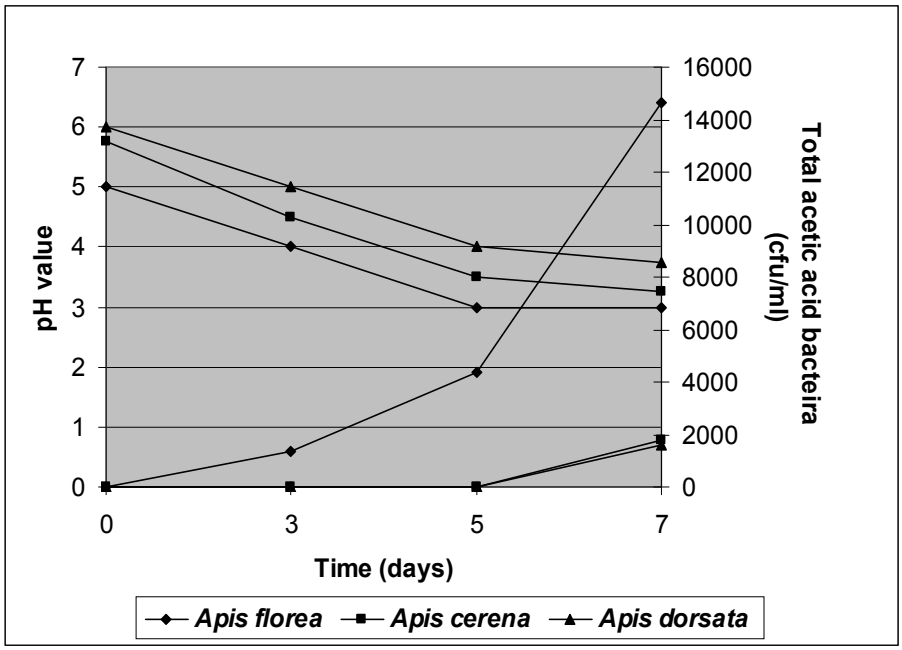

Figure 1. Change in numbers of acetic acid bacteria in culture broth and pH value during enrichment period

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Table 2. Biochemical characterisation of acetic acid bacterial isolates

Biochemical test	CMU1	CMU2	CMU3	CMU4
Overoxidation	-	-	-	-
Catalase	+	+	+	+
Growth in ethanol	+	+	+	+
Production of gluconate	+	+	+	+
Ketogenesis from glycerol	-	-	-	-
Cellulose production	-	-	-	-
Soluble pigment	-	-	-	-

Note: + = positive; - = negative

The four isolates were tested for their ability to grow in broths supplemented with acetic acid and alcohol at 30 and 37°C. It was found that all isolates could grow in 1% (v/v) acetic acid only at 30°C. However, these isolates showed remarkable tolerance to ethanol, particularly isolate CMU4, which was able to grow well in a medium containing as high as 10% ethanol (v/v) at 30°C (Table 3). In general, the alcohol tolerance decreased with higher temperature (Tables 3, 4). It seems that there may be some relationship between thermotolerance, acetic acid resistance and ethanol resistance characteristic in this group of bacteria. This suggestion is supported by the observation that thermotolerant growth in the presence of acetic acid and high ethanol concentration is an outstanding characteristic of acetic acid bacteria from Thailand [11]. However, little has so far been reported about the mechanism of either acetic acid or ethanol resistance. Further investigation has to be done to test this hypothesis.

Table 3. Alcohol tolerance of acetic acid bacterial isolates at 30°C

Isolate	Percentage of alcohol (v/v)				
	6	7	8	9	10
CMU1	++++	++++	++++	+++	-
CMU2	++++	++++	+++	-	-
CMU3	++++	++++	++++	+++	-
CMU4	++++	++++	++++	++++	+++

Note : ++++ = very good growth; +++ = good growth; - = no growth

Surprisingly, in this study, the use of enrichment medium with ethanol which usually favours the development of *Acetobacter* yielded only *Gluconobacter*. This is probably due to the nature of honey with very high concentration of sugar, which allows only the growth of osmotolerant or osmophilic microorganisms including member of the genus *Gluconobacter*. This result is in agreement

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Table 4. Alcohol tolerance of acetic acid bacterial isolates at 37°C

Isolate	Percentage of alcohol (v/v)				
	6	7	8	9	10
CMU1	++++	++++	+++	-	-
CMU2	++++	+++	-	-	-
CMU3	++++	++++	+++	-	-
CMU4	++++	++++	++++	+++	-

Note : ++++ = very good growth; +++ = good growth; - = no growth

with earlier reports by Ruiz-Argueso and Rodriguez-Navarro [4,5] who examined the microorganisms of ripening honey from an apiary in Madrid, Spain. They found that *Lactobacillus viridescens* and *Gluconobacter* were two main groups of bacteria present in honey. These authors concluded that the microbial flora of honey appears to be the result of chance as honey bees come into contact with several microorganisms during their visit to various niches. Though *Gluconobacter* was found in association with the honey bees, it is not known whether the presence of the acetic acid bacteria has any significance for the bees or any hive products including honey [6].

Conclusions

The first isolation of acetic acid bacteria from honey in Thailand was reported in the present study, the results of which have also provided evidence that the enrichment culture technique is useful in promoting the growth of acetic acid bacteria. These bacteria present in honey also show high tolerance to ethanol, which suggests their usefulness in fermentation industry.

Acknowledgement

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Full Paper

Anti-*Aeromonas hydrophila* activity and characterisation of novel probiotic strains of *Bacillus subtilis* isolated from the gastrointestinal tract of giant freshwater prawns

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Abstract: The antagonistic activity of two *Bacillus* strains isolated from the gastrointestinal tract of giant freshwater prawns against *Aeromonas hydrophila* was evaluated in vitro. The characterisation of the novel probiotic strains of these bacilli was also performed. *Bacillus subtilis* P33 and 72 were found to have high inhibition activities against the growth of *A. hydrophila* by two assay methods: paper disc and well diffusion. Probiotic properties, namely acid and bile salt tolerance, autoaggregation, coaggregation, hydrophobicity and adhesion to Caco-2 cells, were further analysed. Survival rates in model gastrointestinal tract condition, viz. pH 2.5 for 3 h and 0.3% bile salt for 24 h, were shown to be more than 95% and 90% respectively. The ability of *B. subtilis* strains of P33 and P72 to adhere to epithelial cells of the host animal was measured by percentage autoaggregation (35.7 and 42.2%), coaggregation (11.1 and 11.6%), hydrophobicity in *n*-hexadencane (25.6 and 30.0%), xylene (32.2 and 36.1%), toluene (30.3 and 31.6%), and adhesion to Caco-2 cells (4.21 and 3.23 log cfu/ml respectively). These results indicate that both strains of *B. subtilis* P33 and P72 can be considered to be good novel probiotic candidates for use in the prawn aquaculture industry.

Keywords: probiotic, giant freshwater prawns, *Bacillus subtilis*, *Aeromonas hydrophila*, Caco-2 cells

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Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of most commercially important food commodities in the world especially in many Asian countries such as Taiwan, Thailand, and Vietnam [1]. In the prawn aquaculture industry, *Aeromonas hydrophila* infection is considered a major cause of shell diseases and low rate of survival [2-3]. The use of antibiotics to prevent these diseases has normally been practiced in many cases although their indiscriminate use has led to increase in antibiotic resistance and residual level in the products [4-6], which has affected the shrimp export of Thailand [7]. Probiotics as microbial cells are administered in such a way as to enter the gastrointestinal tract and to be kept alive, with the aim of improving health [8]. The main purposes of using probiotics in aquaculture were shown to include competitive exclusion of pathogenic bacteria [9-10], as source of nutrients and enzymatic contribution to digestion [11] and enhancement of the immune response against pathogenic microorganisms [10],[12-14]. Although several investigations have reported the case of using potential probiotics in shrimp aquaculture, there seems to be no report of similar cases for the giant freshwater prawn. In this study, therefore, the characteristic activity and antagonistic ability of the novel probiotic strain of *Bacillus subtilis* isolated from the gastrointestinal tract of giant freshwater prawns were investigated.

Materials and Methods

Bacterial strains

The tested strains of *B. subtilis*, P33 and P72, were isolated from the intestines of giant freshwater prawns which were obtained from Chaopraya River, while the compared strain of *B. subtilis*, TISTR 08, and the pathogenic strain of *Aeromonas hydrophila*, TISTR 1321, were purchased from Thailand Institute of Scientific and Technological Research. The tested strains were fundamentally characterised by being gram positive, rod shape, spore former and catalase positive, thus indicating a general morphology or characteristic of *Bacillus* [15]. The strains were maintained at -80^{o} C in 20 % (v/v) glycerol (Merck, Germany) until further analysis.

Antagonistic activity of Bacillus strains against A. hydrophila

Two methods of agar diffusion assays were used in this study. The first was the paper disc diffusion assay, a modification of the paper disc (Durchmesser: 6 mm, Macherey-Nagel, Germany) diffusion method used as triplicate tests. Both groups of the bacterial strains (the tested strains including the compared strain and the pathogenic strain) were briefly grown in a nutrient broth (Merck, Germany), incubated at 37^{o} C for 18 h, and adjusted to an approximate concentration of 10^{8} cfu/ml. Each sterilised paper disc was impregnated with 20 µl of a diluted test bacterial isolate and placed on the surface of an agar plate which was previously inoculated with the indicator pathogen at a concentration of about 10^{7} cfu/ml. The plate was then incubated at 37^{o} C for 24 h and the inhibition zone around paper disc was recorded. The second method was the well diffusion assay in which the nutrient agar (Merck, Germany) plates were each overlaid with 10 ml of molten nutrient broth (Merck, Germany) containing 0.7% agar at 45° C and inoculated with the 18-h culture of the pathogenic strain above to obtain a final concentration of approximately 10^{6} cfu/ml. Upon solidification of both agar

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layers, a sterile cork borer was applied to create wells of 8 mm in diameter. The cell-free supernatant (100 µl) from the broth containing the 18-h culture of the tested strains were transferred into each well and incubated at 37° C for 24 h under aerobic condition. *B. subtilis* TISTR 08 was used as the control. The inhibition of a clear zone around the well showing no growth of the indicator pathogen was recorded. Each sample was done in triplicate.

API 50 CHB assay

The profile of biochemical test of the isolates was evaluated with API 50 CHB (BioMérieux®, France) strips following the manufacturer's instructions. Briefly, bacteria were grown in nutrient broth at 37 °C for 18 h. After centrifugation at 5000 g for 15 min, cells were washed twice with sterile distilled water. The bacteria were adjusted with sterile distilled water to achieve an approximate concentration at 2 McFarland. The bacterial suspension was mixed with API 50 CHB medium and added into the wells of API 50 CHB strips. These suspensions were incubated at 37° C for 24 h. The ability of the bacterial strains to ferment 49 different carbohydrates was used to classify the strains.

Acid and bile salt tolerance assays

Acid tolerance was evaluated using a modified method of Conway et al. [16] and Pennacchai et al. [17]. Cultures were grown in nutrient broth (Merck, Germany) at 37°C for 18 h. One ml of bacterial suspension was transferred into 9 ml of sterile phosphate buffer saline solution adjusted to pH 2.5 with 5 N HCl (Merck, Germany). The initial bacterial concentration was almost 10^7 cfu/ml, which was then incubated at 37°C for 0 and 3 h. Viable bacteria were counted after incubation at 37° C for 24 h on nutrient agar. For bile salt tolerance, the method of Gilliland et al. [18] was performed. One ml of bacterial suspension was inoculated into 9 ml of sterile nutrient broth prepared with bile salt (Sigma, USA). About 0.3% of bile salt concentrate was applied. The suspension was incubated at 37° C on nutrient agar and viable bacteria were counted after exposure of 0 and 24 h.

Autoaggregation and coaggregation assays

Aggregation of bacterial isolates was evaluated by the method of Del Re et al. [19] as modified by Kos et al. [20]. For determination of autoaggregation, the tested bacteria were grown at 37° C for 24 h on a nutrient broth (Merck, Germany). After centrifugation at 5000 g for 15 min, cells were washed twice and resuspended in phosphate buffer saline to give a viable concentration of about 10^7-10^8 cfu/ml. Four ml of the cell suspension were mixed for 10 s to determine autoaggregation during 5 h of incubation at room temperature. The upper suspension was used in each hour by transferring 0.1 ml to another 3.9 ml of phosphate buffer solution, and the optical density at 600 nm was measured. Per cent autoaggregation was calculated by the formula: $1-(A_t/A_0) \times 100$, where A_t represents the absorbance at time t = 1, 2, 3, 4 or 5, and A_0 the absorbance at t = 0. For determination of coaggregation, the cell suspension was prepared similar to the autoaggregation assay. Two 2-ml aliquots of the cell suspensions were mixed together by vortexing for 10 s. About 4 ml of individual cell suspension was set aside as control group at the same time. The absorbance at 600 nm was measured after mixing and incubating at room temperature for 5 h. Coaggregation was calculated according to Handley et al. [21]: $(A_x + A_y)/2 - A_{(x+y)}/A_x + A_y/2 \times 100$, where A_x and A_y represented absorbance of each of the two strains.

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Hydrophobicity assay

Determination of cell surface hydrophobicity was evaluated according to the ability of the microorganisms to partition into hydrocarbon from phosphate buffer solution using the method of Savage [22]. Bacterial isolates were grown in nutrient broth (Merck, Germany) at 37°C for 24 h. After being centrifuged at 5000 g for 15 min, the pellets (bacterial precipitates) were washed twice with phosphate buffer solution and optical density of the bacteria at 450 nm adjusted to 0.5 A. About 1 ml of bacterial suspension was added with 60 µl of a hydrocarbon, viz. *n*-hexadecane (Fluka, Germany), xylene (Fisher, England), or toluene (Merck, Germany), and vortexed for 1 min followed by determination of optical density of the water phase. Hydrophobicity was calculated according to the equation: $[(OD_{450}$ before $- OD_{450}$ after$)/OD_{450}$ before$] \times 100 =$ % hydrophobicity.

Adhesion assay on Caco-2 cells

The method of Gagnon et al. [23] was performed with a little modification. Caco-2 cells were routinely grown in Dulbecco's modified Eagle's minimal essential medium (DMEM, Sigma-Aldrich, USA) supplemented with 10% (v/v) fetal calf serum (Hyclone, USA) inactivated at 56°C for 30 min, 1% (v/v) non-essential amino acids (Hyclone, USA), and 1% (v/v) penicillin-streptomycin (10,000 IU/ml and 10,000 µg/ml; Hyclone, USA). Cells were incubated at 37° C in 5% CO_2 in air. For the bacterial adhesion assay, Caco-2 cells were seeded with 1 ml of culture medium containing 10^5 viable cells/well in 24-well tissue culture plates. The culture medium was changed every 48 h while Caco-2 cells were used at post-confluence after 15 days to become fully differentiated. The medium of non-supplemented DMEM was replaced at least 1 h before adhesion assay. Tested bacteria from the 18-h cultures in nutrient broth (Merck, Germany) were harvested and washed twice with phosphate buffer saline. Cells were resuspended in non-supplemented DMEM to achieve a concentration of 10^8 cfu/ml. After washing the Caco-2 twice with phosphate buffer saline, 0.5 ml of bacterial suspension was added to each well and incubated at 37° C for 1 h in 5% CO_2 in air. Removing unattached bacteria was performed by washing with the sterile phosphate buffer saline 3 times. Caco-2 cells were lysed with 0.1% Triton X-100 (Merck, Germany) for 5 min. The concentration of adhered bacterial cells were enumerated by plate counting in triplicate on nutrient agar and then incubating at 37° C for 24 h. The adhesion of bacterial strains to Caco-2 cells was expressed as log cfu/ml by comparing the initial and viable bacteria in the DMEM suspension. Each adhesion assay was performed in triplicate.

Results and Discussion

The diversity of microbial population in the gastrointestinal tract of prawns had been found in many different prawn species in research findings (data not shown) which showed that the *Bacillus* strains have characteristics of being Gram positive and spore formers having rod shape and the ability to produce catalase enzyme [15]. Both isolates of *B. subtilis* P33 and P72 were found to be catalase positive, an indicator that anaerobic spore-forming *Clostridium* spp. were absent. Barbosa et al. [24] reported that catalase positive property is a characteristic of *Bacillus*, thus separating it distinctly from *Clostridium* spp.

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Based on the recently proposed use of probiotic bacteria to prevent shrimp diseases [25], this study was aimed to identify the novel probiotic strains in order to apply them as a disease control in giant freshwater prawn aquaculture. The characteristics of a successful probiotic consist of antimicrobial activity against intestinal pathogens, acid and bile tolerance, and the ability to adhere to and colonise the intestinal tract [26-27]. The determination of the antimicrobial activity of *B. subtilis* P33 and P72 which were isolated from the gastrointestinal tract of giant freshwater prawns against *A. hydrophila* was performed by paper disc and well diffusion assay. The antagonistic effect of these isolates on the growth of indicator pathogen could be determined by the appearance of clear inhibition zones around the paper disc or well (Figure 1 and Table 1). Previous studies showed that *Bacillus* species could produce a large number of antimicrobials [28]. In addition, the cell-free extracts of *B. subtilis* BT23 showed greater inhibitory effects against the growth of *V. harveyi* which was isolated from the black gill disease of *Penaeus monodon* [29]. The *B. subtilis* UTM 126 possessed an antimicrobial activity against pathogenic *Vibrio* strains that included *V. alginolyticus*, *V. parahaemolyticus* and *V. harveyi* [25]. All these suggest that the antimicrobial-producing strains of *B. subtilis* P33 and P72 may play an important role in suppressing the growth of harmful *A. hydrophila*.

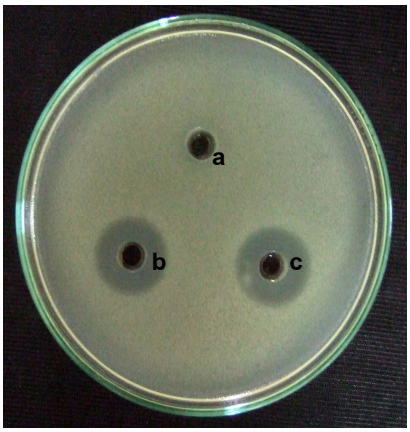

Figure 1. Agar well diffusion assay showing antagonistic activity of *Bacillus* strains against *A.hydrophila* TISTR 1321: (a) *B. subtilis* TISTR 08, (b) *B. subtilis* P33, and (c) *B. subtilis* P72

Table 1. Antagonistic activity of *Bacillus* strains against *A. hydrophila* TISTR 1321

Bacteria	Inhibition zone (mm.)	
	Paper disc diffusion	Well diffusion
B. subtilis TISTR 08	-	-
B. subtilis P33	14.5±0.5	18.3±0.6
B. subtilis P72	13.7±1.3	19.0±1.0

Note: - = no inhibition

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The method using biochemical technique was applied in identifying the type of *B. subtilis* P33 and P72. These isolates were subjected to sugar fermentation pattern analysis by API 50 CHB test strip. Exhibiting a rate of 96.6%, both P33 and P72 isolates were identified as belonging to the species of *Bacillus subtilis*.

In order to survive in the gastrointestinal tract, a probiotic candidate must be resistant to the salivary enzyme, gastric acid and bile, and able to establish itself in the intestinal microbiota. The tolerance of both *B. subtilis* P33 and P72 strains to acid (pH 2.5) and bile salt (0.3%) were reported as % survival rate (Figure 2). The low pH tolerance of both strains was shown to be more than 95%. The growth of these strains in nutrient broth containing 0.3% bile salt after 24-h incubation indicated a high rate of tolerance of more than 90% for both strains. Previously, probiotic strains of *Bacillus* species and *B. subtilis* MA 139 were shown to exhibit resistance to bile salts and simulated gastric conditions [30], and in fact, some *Bacillus* species were frequently found in the intestinal tract [24,31]. These findings suggest that both of these probiotic candidates could survive transit through the gastrointestinal tract and establish themselves in the intestinal environment in which they may cause effective action.

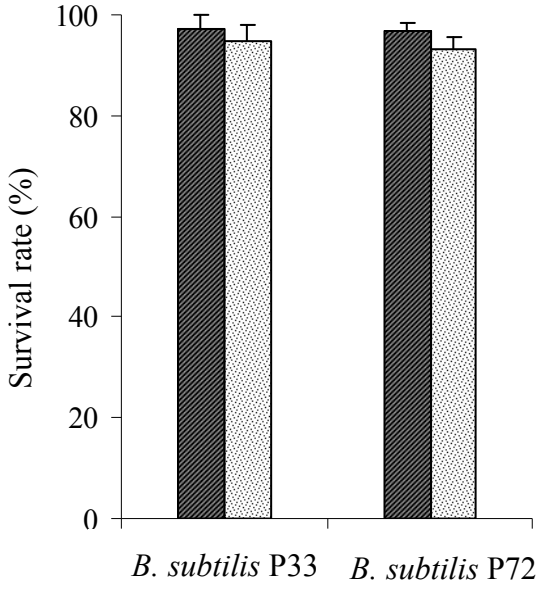

Figure 2. Tolerance of *B. subtilis* P33 and P72 strains to ▨ acid (pH 2.5) and ▩ bile salt (0.3%)

The autoaggregation percentage of the tested isolates was determined during a period of 5 h (Table 2). In the beginning, the percentage of autoaggregation ranged between 10.5–14.2%, and then continually increased every hour. In the final 5th hour, the autoaggregation registered a high percentage of 35.7–42.2. Coaggregation of these isolates with *A. hydrophila* was expressed as per cent reduction in the absorbance of a mixed suspension after 5 h. The rates of both isolates were 11.1 and 11.6% for *B. subtilis* P33 and P72 respectively. Previous studies showed that the property of aggregation is related to cell adherence and interacts closely with undesirable bacteria [19,32]. The strains with the highest autoaggregation or coaggregation were selected for further tests in probiotic screening steps [33]. The method of coaggregation with gut pathogens may be useful for screening to identify potential probiotic

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strains [20,34]. Both of the tested strains in this study exhibited high autoaggregation and moderate coaggregation. A similar result was observed in which a probiotic strain of *L. acidophilus* M92 showed a high score in autoaggregation but lower score in coaggregation with pathogens [19]. The inhibitor producing lactic acid bacteria which coaggregate with pathogens may constitute an important host protective mechanism against infection in the urogenital tract [35], as well as in the gastrointestinal tract [36].

The use of *n*-hexadencane, xylene, and toluene to evaluate the hydrophobic cell surface properties of the tested *Bacillus* isolates showed a rather consistent result. The hydrophobicity of *B. subtilis* P33 and P72 strains was 25.6–30.0 % in *n*-hexadecane, 32.2–36.1 % in xylene, and 30.3-31.6% in toluene (Table 2). Surface hydrophobicity was determined in order to test for possible correlation between this physico-chemical property and the ability to adhere to the intestinal mucus as suggested [37].

Table 2. Adhesion property of *B. subtilis* P33 and P72 by different testing methods: hydrophobicity, autoaggregation and coaggregation

Bacteria	Hydrophobicity (%)			Aggregation (%)					
				Auto- (h)					Co-
	Hexadec-ane	Toluene	Xylene	1	2	3	4	5	
B. subtilis P33	25.6±1.6	30.3±9.4	32.2±5.6	14.3±0.0	21.4±10.1	35.7±10.1	35.7±10.1	35.7±10.1	11.1±0.0
B. subtilis P72	30.0±2.6	31.1±3.7	36.1±2.0	10.6±0.8	36.7±4.7	47.2±3.9	41.7±11.8	42.2±3.1	11.7±3.7

Adhesion to the intestinal epithelium and mucus is found to be associated with stimulation of the immune system [38-39], and adhesion to the intestinal mucosa is also crucial for transient colonisation [39], an important prerequisite for probiotics to control the balance of the intestinal microbiota [40]. The ability to adhere to the intestinal mucosa is therefore an important criterion for in vitro probiotic selection [41], hence the use of Caco-2 cells in this study. The result indicated that both of the two tested strains could adhere to Caco-2 cells. The adhesion to Caco-2 cells was 4.21 and 3.23 log cfu/ml for *B. subtilis* P33 and P72 respectively (Figure 3). Similar results were found using several other species such as *Lactobacillus* sp. [42], *L. casei rhamnosus* [43], *L. rhamnosus* DR20 and *Bifidobacterium lactis* DR10 [44], *L. fermentum* [45], and *L. plantarum* [46]. These findings suggest that both *B. subtilis* P33 and P72 strains have the ability to adhere to the epithelial cells of the host animals.

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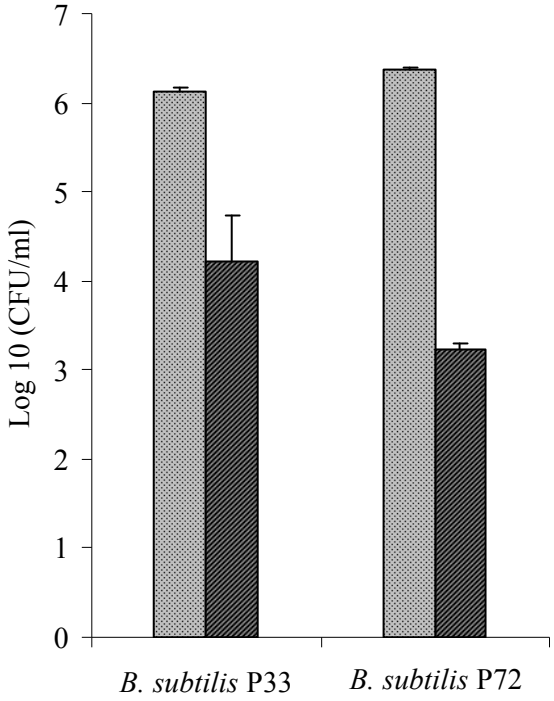

Figure 3. Adhesion of *B. subtilis* P33 and P72 to Caco-2 cells for initial bacterial (▩) and adhered bacterial (▨) concentration

Conclusions

The *B. subtilis* strains of P33 and 72 which originally came from the gastrointestinal tract of the giant freshwater prawn, were found to show inhibiting activities against the growth of *A. hydrophila*. The probiotic property of both strains could survive in acidic medium (pH 2.5) and 0.3 % bile salt solution. The two strains also exhibited ability to adhere to epithelial cells as shown by aggregation, hydrophobicity and adhesion to Caco-2 cells, thus indicating that they could be considered as good novel probiotic candidates for use in the prawn aquaculture industry.

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Full Paper

Occurrence of potentially pathogenic *Vibrio* species in raw, processed, and ready-to-eat seafood and seafood products

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Abstract: This study investigated the occurrence (by means of the presence-absence test) and level (by means of a plate count technique) of selected potentially pathogenic *Vibrio* species in processed and ready-to-eat seafood, and some raw seafood normally used as raw materials or ingredients in these products, that were commercially available in Chiang Mai, Thailand. The level of *Vibrio* in raw seafood was found to range from 50 to 10^4 cfu/g. *V. alginolyticus* was the most frequently found species, followed by *V. parahaemolyticus*, *V. cholerae*, *V. mimicus*, and *V. vulnificus*, in that order. Processed and ready-to-eat products were contaminated with at least one of the potentially pathogenic vibrios at significant frequencies (25 and 17.5 % of samples, respectively), with the level as high as 10^3 to 10^4 per gram in some samples. Incidences of vibrios revealed by the presence-absence test were significantly higher than those revealed by the plate count assay. These data point to the hazard potential relating to *Vibrio* in processed and ready-to-eat seafood and the need to strictly apply preventive measures against *Vibrio* gastroenteritis through consumption of these food products. They also suggest that analytical methods used in food safety evaluation in relation to potentially hazardous *Vibrio* species should be carefully considered.

Keywords: *Vibrio* species, food safety, seafood, seafood products, ready-to-eat seafood

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Introduction

Vibrio species, Gram negative rod- (or curved rod-) shaped bacteria, are known to occur naturally in marine and freshwater environments and thus are commonly associated with seafood and/or food of freshwater origin [1-3]. Many species can cause gastrointestinal diseases. *V. parahaemolyticus* has been frequently involved in outbreaks of foodborne diseases worldwide [4,5]. *V. cholerae* also constitutes a very important risk. The serogroups O1, O139, and O141 cause cholera, while other serogroups can cause less severe diarrhea [5-7]. *V. vulnificus* is another organism of great concern in seafood safety due to the severity of the disease and the high mortality rate it can cause [1,8-9]. Other species that have been increasingly recognised as food pathogens in recent years are *V. mimicus* and *V. alginolyticus*. *V. mimicus* has genetic and many biochemical similarities to *V. cholerae* [10,11], and its pathogenicity involves several toxins including that of *V. cholerae* [12]. Many foodborne outbreak cases involving *V. mimicus* have been reported [13-15]. *V. alginolyticus* is one of the most common *Vibrio* species occurring in marine environments and seafood [3,16-19]. This species is an opportunistic pathogen [20,21] and its pathogenicity is thought to be similar to that of *V. parahaemolyticus* [22].

The occurrence of *Vibrio* spp. in raw seafood is common, especially seafood from regions with temperate climates around the world, from both natural and farm environments, and in seafood of all types [16-19,23-27]. However, most surveys are qualitative, which causes difficulties in evaluating the risks relating to *Vibrio* spp. in raw seafood. The level of *Vibrio* spp. in raw seafood can also affect survival of the organisms through processing. For processed and ready-to-eat seafood (including ready-to-eat products that are intended for raw consumption, such as raw oyster [28,29]), the presence and level of *Vibrio* spp. has a direct impact on food safety. Cases of foodborne outbreaks resulting from consumption of ready-to-eat seafood dishes, especially those supplied by food catering/food service establishments, continually occur [30-34]. Nevertheless, reports on occurrence of *Vibrio* spp. in processed and ready-to-eat seafood are scarcely available. A few examples are the incidence of *V. parahaemolyticus* in smoked fish [35] and cooked crayfish [36].

The scarce availability of quantitative data for *Vibrio* spp. in seafood and of information about the occurrence of *Vibrio* spp. in processed and ready-to-eat seafood has set the interest for this study. We aim to examine potentially pathogenic species listed above in raw, processed, and ready-to-eat seafood and seafood products by means of qualitative and quantitative methods. Data obtained from this study should benefit food catering/food service establishments, food safety-related personnel and authority, and those involved in food industry.

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Materials and Methods

Food samples

A total of 118 seafood samples or samples containing seafood were randomly collected in Chiang Mai, Thailand. These samples were commercially available in food markets or supermarkets, or prepared by food catering/food service establishments. They consisted of 39 raw seafood samples (squids, crustaceans, fish and shellfish), 16 industrial-scale processed seafood products (seasticks, fish/shrimp balls, fish noodles, fish tofu, squid rolls), and 63 ready-to-eat seafood dishes (sushi-type meals; grilled, fried, steamed, shortly boiled seafood; and seafood-containing dishes prepared by various cooking methods).

Preparation of food samples for analysis of Vibrio *spp.*

Food samples were transferred to the laboratory in closed, sterile containers under cooled conditions. A sample was cut aseptically into small pieces (approx. 0.5-1.0 × 0.5-1.0 cm) and prepared in two separate 25-gram portions for the qualitative (presence-absence) and quantitative (plate count) analyses. For shellfish, the shells were separated and only the flesh was used in analysis. For large-size seafood such as crab or fish, pieces from all different parts were taken for a sample. The food samples were analysed within 2 hours after collection.

Presence-absence analysis and enumeration of Vibrio *spp. in seafood*

Analysis of *Vibrio* spp. (*V. cholerae, V. parahaemolyticus, V. mimicus, V. vulnificus* and *V. alginolyticus*) in seafood was carried out using a method modified from that described in the Bacteriological Analytical Manual [37]. In brief, for presence-absence analysis, a 25-gram portion of a food sample was homogenised in 225 ml of alkaline peptone water (APW, prepared from bacteriological peptone supplied by Hi Media, India), for 1 min using a food homogeniser (Seward Stomacher 400, Brinkmann, Canada). The homogenate was then incubated at 37 ºC for 18 h. This was then transferred to streak on thiosulphate-citrate-bile salt-sucrose (TCBS, Hi Media, India) agar plate, followed by incubation at 37 ºC for 18-24 h. For enumeration, the other 25-gram portion was homogenised in 0.1 % peptone water in the same manner, giving a 10^{-1} dilution, which was further diluted in a 10-fold series until desired dilutions were obtained. The homogenate (0.1 ml) was then surface-spread on TCBS agar plates in duplicate. The inoculated TCBS plates were incubated at 37 ºC for 18-24 h, and a presumptive count was made for each colony type. For both procedures, at least five representative colonies (or all colonies if less than five were recovered) of each colony type were collected and subjected to biochemical tests (sodium chloride tolerance (0-10 %) and lactose utilisation), leading to differentiation of *Vibrio* species according to the species characteristics of human pathogenic Vibrionaceae commonly encountered in seafood listed in the Bacteriological Analytical Manual [37]. The bacterial isolates were maintained on trypticase soya agar (TSA, Hi

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Media, India) slant containing 1.0 % sodium chloride at 4 ºC or as stab cultures in the same medium at room temperature.

Reporting the occurrence and level of Vibrio *spp.*

The results from the qualitative analysis for each *Vibrio* spp. in a food sample was reported as being present or absent in 25 grams of food. From the quantitative analysis, the level of each *Vibrio* spp. in colony forming units per gram (cfu/g) was deduced as followed.

$$\text{Level of a certain } \textit{Vibrio} \text{ species (cfu/g)} = \frac{(R2/R1)\times T}{W}$$

where: R1 = number of representative presumptive colonies (cfu) sampled for analysis
R2 = number of representative colonies (cfu) identified as a certain species
T = Total number (average from duplicates) of presumptive colonies (cfu) on TCBS agar plates of selected dilution
W = weight (gram) of food sample in analytical volume of food homogenate drawn from the dilution in which enumeration was performed

Results and Discussion

The qualitative (presence–absence test) and quantitative (plate count) methods were used in parallel in order to obtain the advantages offered by both. The presence–absence test is more sensitive in revealing the presence of the organisms while the plate count method gives the level of contamination which is more closely related to illnesses potentially caused by *Vibrio* species. The analysis of seafood or seafood-containing samples by the presence-absence test and/or plate count revealed contamination of different potentially pathogenic *Vibrio* spp. in all seafood categories (raw, processed, and ready-to-eat).

Contamination of raw seafood by *V. alginolyticus* was most frequent (61.5 %), followed by *V. parahaemolyticus* (43.6 %), *V. cholerae* (35.9 %), *V. mimicus* (23.1 %), and *V. vulnificus* (2.6 %). The level of contamination ranged from 50 to 4.5×10^4 per gram (Table 1). Since *Vibrio* spp. can occur naturally in an aquatic environment, the presence of these organisms in raw seafood may be expected [38,39]. However, the high level (10^3-10^4 per gram) of *Vibrio* spp. in some samples of raw seafood may indicate inadequate control in storage temperature from the time of harvesting, and this level is regarded as unsatisfactory by some food criteria [39]. Furthermore, the high level (up to 10^4 per gram) of *V. parahaemolyticus* (such as that found in clam, Table 1) is regarded as potentially hazardous [39], considering the possibility of the contaminant strain(s) being pathogenic. These potentially pathogenic vibrios would also have an impact on safety of processed/cooked ready-to-eat food if they survive

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insufficient processing/cooking conditions, or they could be an important source of recontamination after processing.

Table 1. Levels of contamination of potentially pathogenic *Vibrio* spp. in raw seafood

Seafood group	Level of contamination by potentially pathogenic species				
	V. alginolyticus	*V. cholerae*	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*
squid	1.0×10^{2}-3.0×10^{4}	3.0×10^{3}-7.0×10^{3}	1.0×10^{2}-9.5×10^{3}	1.0×10^{3}-2.0×10^{3}	1.0×10^{2}
prawn, shrimp, lobster	1.5×10^{2}-1.0×10^{3}	1.8×10^{3}-5.6×10^{3}	not detected	5.0×10^{1}-4.0×10^{2}	not detected
shellfish (clam, mussel, oyster)	1.5×10^{2}-4.5×10^{4}	3.9×10^{3}	1.0×10^{3}-3.3×10^{4}	1.0×10^{2}-1.3×10^{4}	not detected
crab	1.3×10^{3}-2.8×10^{3}	1.6×10^{4}	1.0×10^{2}	1.8×10^{3}-6.7×10^{3}	not detected
fish	6.7×10^{2}-3.5×10^{3}	3.3×10^{2}	2.0×10^{2}	1.1×10^{3}	not detected

Contamination of pathogenic *Vibrio* spp. in industrially processed seafood products and ready-to-eat seafood dishes is demonstrated in Table 2. The summary of the overall positive results obtained by the presence-absence and plate count analyses are given in Table 3.

From Tables 2 and 3, industrially processed and ready-to-eat seafood samples were contaminated with the potentially pathogenic *Vibrio* spp. at significant frequencies. The presence of *V. cholerae* and *V. parahaemolyticus* (as well as other vibrios) in industrially processed and ready-to-eat foods is contrary to what is expected [39-41]. This also applies to other vibrios. Industrially processed seafood products, such as fish/shrimp balls and sea sticks, generally are passed through a pasteurisation process, which should eliminate all non-spore-forming microorganisms. As for the ready-to-eat seafood dishes examined, most were cooked dishes, except one that was intended for raw consumption (prawn in fish sauce). The occurrence of *Vibrio* spp. in processed and cooked ready-to-eat seafood indicated insufficient processing or post-process contamination.

As summarised in Table 3, the proportion of samples contaminated with *Vibrio* spp. as determined by the presence-absence and the plate count method was significantly different. The presence-absence analysis was more sensitive, revealing more positive samples than the plate count analysis, as expected. This stresses the importance, even when quantitative data are required, of applying the presence-absence analysis parallel to enumeration in examining vibrios in seafood for compliance with a zero-tolerant standard. This is crucial, especially when analysing processed food in which low number of organisms are expected, food which has potential to allow low level of *Vibrio*

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contaminants to multiply, food prepared for the immunocompromised group, and food suspected of containing *Vibrio* spp. of severe hazard category or those which have a low infectious dose.

Table 2. Occurrence and level of *Vibrio* spp. in raw seafood, industrially processed seafood products, and ready-to-eat seafood dishes

Sample category	Food sample contaminated with potentially pathogenic *Vibrio* spp.	*Vibrio* species isolated	Occurrence of *Vibrio* spp. in 25 grams of food [a]	Level of *Vibrio* spp. (cfu/g) [b]
industrially processed products	fish balls	*V. cholerae*	P	-
		V. mimicus	P	-
	sea sticks	*V. mimicus*	P	-
	fish balls	*V. cholerae*	P	-
	shrimp balls	*V. parahaemolyticus*	P	-
ready-to-eat seafood dishes	mixed seafood salad	*V. alginolyticus*	P	-
	spicy mackerel salad	*V. alginolyticus*	P	2.02×10^{4}
	prawn salad in lime dressing	*V. mimicus*	P	1.1×10^{3}
		V. cholerae	P	1.0×10^{2}
	steamed seafood in coconut sauce	*V. cholerae*	P	-
	deep-fried battered squid	*V. cholerae*	P	-
		V. mimicus	P	-
	steamed squid in lime sauce	*V. cholerae*	P	-
		V. mimicus	P	-
	squid salad with lime dressing	*V. mimicus*	P	-
	stir-fried prawn with black pepper	*V. cholerae*	P	-
	spicy seafood salad	*V. mimicus*	P	3.9×10^{3}
		V. cholerae	P	5.0×10^{1}
	prawn in fish sauce	*V. cholerae*	P	2.3×10^{3}
	hot and sour soup with prawn	*V. mimicus*	P	-

[a] Presence and absence of *Vibrio* spp. are indicated by P and A, respectively.

[b] The – symbol represents no occurrence by means of surface-spread plate count method, i.e. a level under limit of detection (50 cfu/g).

Since contamination of vibrios in seafood is a problem worldwide (as reviewed above) and the same is assumed for processed and ready-to-eat seafood products, the results of this study are therefore believed to be implicative also for geographical areas other than Thailand. The occurrence or level of the potentially pathogenic *Vibrio* species presented here indicates risks in consumption of undercooked or re-contaminated processed and ready-to-eat (including uncooked) seafood and reaffirms the need to enhance their safety quality. The safety of industrially processed seafood and ready-to-eat seafood

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available at markets/supermarkets or food catering establishments can be of great significance for public health [32,42]. Preventive measures such as proper handling and storage of raw seafood, effective reduction of *Vibrio* spp. in seafood used as raw material, and strict control of safety quality along food processing and food preparation processes (especially in the food industry and food catering unit) should be urgently applied. To promote the safety of ready-to-eat food, of which quality monitoring cannot be carried out routinely, educating food service personnel seems to be the most promising solution.

Table 3. Frequency of positive samples with *Vibrio* spp. revealed by presence-absence and plate count analyses

Seafood category (no. of samples examined)	Number (percentage frequency) of contaminated samples by presence-absence analysis						Number (percentage frequency) of contaminated samples by plate count analysis					
	All vibrios[a]	Specific *Vibrio* species[b]					All vibrios[a]	Specific *Vibrio* species[b]				
		Va	Vc	Vm	Vp	Vv		Va	Vc	Vm	Vp	Vv
industrially processed seafood (16)	4 (25.0)	0 (0)	2 (12.5)	2 (12.5)	1 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ready-to-eat seafood (63)	11 (17.5)	2 (3.2)	7 (10.8)	6 (9.2)	0 (0)	0 (0)	4 (6.3)	1 (1.6)	3 (4.8)	2 (3.2)	0 (0)	0 (0)

[a] all of the 5 species examined.

[b] Va = *V. alginolyticus*, Vc = *V. cholerae* , Vm = *V. mimicus* , Vp = *V. parahaemolyticus* , Vv = *V. vulnificus*.

Conclusions

This study has demonstrated the hazard potential of raw seafood, industrial-scale processed seafood products, and ready-to-eat seafood dishes prepared by food service establishments in relation to potentially pathogenic *Vibrio* spp., which calls for attention and preventive action of the food processing industry, food catering industry, and food authority. It also raises a critical issue in food analysis and standard compliance, as significant differences in the ability of qualitative and quantitative methods to recover *Vibrio* spp. in seafood samples have been demonstrated. The level of *Vibrio* spp. in seafood given here should also be useful for risk assessment concerning pathogenic vibrios.

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Short Report

Database of bryophytes and their ecological parameters in the CMU Herbarium

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Introduction

Chiang Mai University (CMU) Herbarium was originally established in July 1987 at the Faculty of Pharmacy, Chiang Mai University by James F. Maxwell. This herbarium, with over 10,000 specimens of vascular plants and about 40 bryophytes, was discontinued in late 1991. Maxwell subsequently joined the Biology Department, Faculty of Science in March 1992 and developed another collection which now (July 2008) has nearly 30,000 specimens of vascular plants. The Bryophyte Section was started by Dr. Kanya Santanachote in 1999 and has 2,350 specimens.

Bryology Studies in Thailand

The first bryophytes collected in Thailand were from Koh Chang (Chang Island), Trat Province during 1899-1902. V. F. Brotherus [1] prepared a list of Bryales which included 44 species, 18 of which were new species. F. Stephani [2] identified the Hepaticae which had 17 species, 4 of which were new to science. Stephani and Brothereus identified the Hepaticae collected by Hosseus [3] which had 4 new species, on the summits of Doi Sutep-Pui, Chiang Mai during 1904-05. Dixon [4] provided the first list of Thai mosses which consisted of 220 species. The information for most entries comprised the location, elevation, basic habitat, date of collection, and overall distribution. The collections of Dr. A.F.G. Kerr, who collected extensievely in Thailand, were included. Tixier and

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Smitinand [5] provided a detailed list of bryophytes in the Forest Herbarium, Bangkok, which included location, elevation, habit, habitat, and overall geographical distribution. No notes concerning sporophyte or gametophyte stages, abundance, and microhabitats were provided. A concise checklist of Thai bryophytes was published by Sornsamran and Thaithong in 1995 [6]. This book includes the location and publication report information for each species. All other information concerning habitat, elevation, etc. is excluded. He [7] has provided the most recent and reliable list of mosses for Thailand. This essential reference also includes illustrations, references, distributions in Thailand and Asia, elevations, as well as Thai specimens and the herbaria they were deposited in. A total 52 families, 192 genera, 620 species, and 30 subspecific taxa are presented. Lai et al. [8] compiled a list of Thai liverworts and hornworts, which, unfortunately, lacks ecological information. There are 37 families, 90 genera, and 386 species included in this vital publication.

The database presented here (Appendix 1) is the first of its kind for Thai bryophytes. It follows a general format which has been adopted by the CMU Herbarium [9,10]. Forest types and habitats are according to Maxwell [11].

Summary

The Bryophyte Section has more than 2,350 specimens, and is identified into 60 families, 135 genera, 272 species, 4 subspecies, and 9 varieties (Table 1).

Table 1. Bryophytes in the CMU Herbarium

Class	Families	Genera	Species	Sub-species	Var-ieties
Bryopsida	35	94	194	1	9
Hepaticopsida	22	37	69	2	0
Anthocerotosida	3	4	9	1	0
Total	60	135	272	4	9

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We would like to thank Prof. Benito C. Tan and Sutchit Manachit for not only collecting many specimens with accurate notes, but also helping in identification. J. F. Maxwell, curator of the CMU Herbarium, is thanked for suggesting, encouraging, and providing constant assistance in the preparation of this database.

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Appendix 1. Database of bryophytes and their ecological parameters in the CMU Herbarium

Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Bryopsida Sphagnaceae *Sphagnum cuspidatulum* C. Muell.	ac	pe	ter	4	egf	2,556	o,m,c	gr	?	ja-dc	Wong.703	g	ak
Sphagnum luzonense Warnst.	ac	pe	ter	3	egf	1,284	o,s,m	gr	?	ja-dc	Wong295	g	pk
Sphagnum perichaetiale Hampe	ac	pe	ter,emr	3	dof	525	o,m	ss	?	ja-dc	Maxw. B186	g	na
Sphagnum robinsonii Warnst.	ac	pd	epl	3	mxf	1,500	o,s,m	gr	?	ja-dc	Korn.270	g	dl
Archidiaceae *Archidium* sp.	ac	a	ter	4	mxf	730	o,d	gr	?	ja-dc	Prin.27	g	mt
Diphysciaceae *Diphyscium* sp.	ac	pe	epl	2	egf	2,500	s,m	gr	?	ja-dc	San.0515	g	ak
Fissidentaceae *Fissidens aereolatus* Griff.	ac,cuc, ere	pd	ter	4	egf	2,500	s,m	gr	ag-dc	ja-dc	Wong.304,313,314,3217 64,765	g,s	kp,pk
Fissidens anomalus Mont.	ac	pd	cor	4	egf	1,636-2,560	s,m	gr	?	ja-dc	Wong.273,290,709,712,7 49	g	ak,kp, dp
Fissidens backettii Mitt.	ac,cuc, inc	a	ter	2	mxf	1,100-1,300	s,m	gr	jl-oc	ja-dc	Wong.150,155,338,339,3 40,342	g,s	pk,ml, dp
Fissidens bryoides Hedw. subsp. *schmidii* (C. Muell.) Nork.	ac	pd	epl	3	egf	1,355	s,m	gr	jl-nv	ja-dc	Korn.195	g,s	ck
Fissidens ceylonensis Dozy & Molk.	ac,cuc, ere	pd	ter, epl, cor	5	mxf	730-850	o,d, s, m	gr	jn-oc	ja-dc	Prin.41,83,108, Polb.17,51,79,Man.11,20 3,228	g,s	mt,so

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Fissidens crassinervis Lac. var. *laxus* (Sull. & Lesq.) Eddy	ac,cuc, ere	pd	ept	3	mxf,egf	1,010	s,m	gr	ag-dc	ja-dc	San.209	g,s	dp
Fissidens crenulatus Mitt. var. *elmeri* (Broth.) Z. Iwats & Suzuki	ac	a	ter,cor	1	dof,mxf	1,100	o,d	gr	?	ja-dc	Wong.173,181,405,553,612,644	g	pp,cd, nn,dp, ky
Fissidens crispulus Brid. var. *crispulus* Musc.	ac,cuc, ere	pd	ter	5	mxf,egf	730-1,360	s,m	gr	jn-oc	ja-dc	Prin.42,59,61,89, Polb.3,16,39,63, Prom.24,Korn.242	g,s	mt,ck
Fissidens crispulus Brid. var. *robinsonii* (Broth.) Z. Iwats.& Z.-H.Li	ac,cuc, ere	pd	ter, cor	3	mxf	730	s,m	gr	?	ja-dc	Prin.46,69,77, Polb.30,40,66, Prom.22	g,s	mt
Fissidens flaccidus Mitt.	ac	pd	ter	3	mxf	850	o,m	gr	?	ja-dc	Man.172,173	g	so
Fissidens gangdongensis Z. Iwats. & Z. H. Li	ac	a	ter,cor	3	mxf	1,100-1,300	o,m	gr	?	ja-dc	Wong.158,303,310,330	g	pk,ml
Fissidens geminiflorus Dozy & Molk.	ac	ped	epl	3	mxf,egf	730-1,360	s,m	gr	?	ja-dc	Prom.3,28,63,Korn.255	g	mt,ck
Fissidens hollianus Dozy & Molk.	ac,cuc, ere	pd	ter	4	mxf	570-2,500	dof,mxf, egf	gr,ls	jl-oc	ja-dc	Wong.206-209,216,217,221,428440,623,748	g,s	ky,kp, pp,dp, cd
Fissidens incognitus Gang.	ac,cuc, ere	a	ter	1	mxf	730	s,m	gr	?	my-dc	Prin.21,38	g,s	mt
Fissidens javanicus Dozy & Molk.	ac	pd	ter	3	mxf,egf	1,300	s,m	gr	?	ja-dc	Wong.331	g	pk
Fissidens microcladus Thw. & Mitt.	ac	a	cor	3	dof,mxf, egf	350-1,300	o,s,d	g	?	ja-dc	Wong.178,270,271,309,389,391,398,401	g	php, cmu,nl,pk
Fissidens nobilis Griff.	ac	pd	emr, rhe,ter	2	mxf,egf	730-1,355	s,m	gr	?	ja-dc	Prin.71, Prom.1,36, Man.42,Korn.75,100,104,154,156, 184,193,244	g	mt,so, ck

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Fissidens pellucidus Hornsch.	ac	a	ter	2	mxf	730-850	s, m	gr	oc-ja	my-sp	Prin.33,Man.311,314	g,s	mt
Fissidens semperfalcatus Dix.	ac	a	ter	2	mxf	730	s,m	gr	?	my-sp	Prin.44,Polb.31,67	g	mt
Fissidens serratus C. Muell.	ac	a	ter	2	mxf,egf	1,300-2,556	s,m	g	?	ja-dc	Wong.154,336,436,437,438,440	g	pk,dp, ak
Fissidens subbryoides Gang.	ac,cuc, ere	a	ter	2	dof,mxf	570-1,300	o,m	gr	jl-nv	my-dc	Polb.40,41,78,Wong.193, 339,438	g,s	mt,ky, dp,pk
Fissidebs taxifolius Hedw.	ac,cuc, inc	pd	ter,epl	3	egf	1,360	s,m	gr	oc-dc	ja-dc	Korn.74,218,234,244,245	g,s	ck
Fissidens virens Thw. & Mitt.	ac	a	ter	1	mxf	1,200	o,m	gr	?	ja-dc	Wong.162	g	ml
Fissidens zollingeri Mont.	ac,cuc, ere	a	ter	3	dof,mxf, egf	30-1,380	s,m	gr	?	my-sp	Prin63,Polb.34,77,Korn. 223,Maxw.B178	g,s	mt,ck,mk
Ditrichaceae *Ditrichum heteromallum* (Hedw.) Britt.	ac,cuc, ere	pd	epi	3	egf	1,685	s,m	gr	sp-dc	ja-dc	San.192	g,s	dp
Garckea flexuosa (Griff.) Margard. & Nork	ac,cuc, ere	pd	ter	4	mxf,egf	730-1,685	o,d	gr	jn-oc	ja-dc	Prin.49,65,109 Polb.4,15,47,73, Man.151,155,195,216,219, San.200,349	g,s	mt,so, dp
Garckea phaseoides C. Muell.	ac,cuc, ere	pd	ter	3	egf	1,300	s,m	gr	sp-dc	ja-dc	Maxw.B126	g,s	dn
Trematodon sp.	ac	pd	ept	3	mxf	680	s,d	gr	?	ja-dc	San.344	g	dp
Dicranaceae *Brothera leana* (Sull.) C. Muell.	ac	pd	epi	3	egf	1,685	s,m	gr	?	ja-dc	San.150	g	dp
Campylopodium sp.	ac	pd	ept,ter	3	egf	1,300-1,685	s,m	gr	?	ja-dc	San.265,Maxw.B126	g	dp,dn

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Campylopus aureus Bosch *et* Lac.	ac	pd	epi	3	egf	1,030	s,m	gr	?	ja-dc	San.216	g	dp
Campylopus ericoides (Griff.) Jaeg.	ac	pd	ter	4	mxf	730-850	o,d	gr	?	ja-dc	Prin.60,86, Polb.42,64, Man.72,118,236,247	g	mt,so
Campylopus hemitrichius (C. Muell.) Jaeg.	ac	pd	cor	2	egf	1,325	s,m	gr	?	ja-dc	Wong.64	g	km
Campylopus subluteus (Mitt.) Jaeg.	ac	pd	ept	3	dof	500	s,m	gr	?	ja-dc	San.201	g	dp
Dicranella sp.	ac,cuc, ere/inc	pd	ept,ter	3	egf,da	1,550-1,685	s,m	gr	sp-dc	ja-dc	San.4,87,Schw.3	g,s	dp,akh
Dicranodontium uncinatum (Harv.) Jaeg.	ac	pd	ram	3	egf	2,500	s,m	gr	?	ja-dc	San.054/2	g	ak
Dicranoloma fragile (Hook.) Broth.	ac	pd	cor	3	def,egf	1,325-1,685	o,s,m	gr	?	jn-oc	Korn.324,325,326,382,385,386,Wong.65,San.97,125,133,135,156,224	g	sk,km, dp
Leucoloma mittenii Fleisch.	ac,cuc, ere	pd	ter, cor	3	mxf,egf	730-1,325	s,m	gr	ag-nv	ja-dc	Prin.70,Man.27,37,107,130,334,Wong.66,Maxw. B193	g,s	mt,so, km,ky
Microdus sp.	ac	pd	ept,ter	3	egf,mxf	1,320-1,050	s,m	gr	sp-dc	ja-dc	San.180,Maxw.B161	g,s	dp,mm
Wilsonniella decipens (Mitt.) Alst.	ac,cuc, ere	pd	ter	3	dof	700	o,m	gr	ag-dc	ja-dc	Prin.137	g,s	hk
Leucobryaceae *Leucobryum aduncum* Dozy & Molk. var. *scalare* C. Muell. *ex* Fleisch.) Eddy	ac,cuc, inc/hor	ped	cor, ram, lig	4	mxf,def	730-1,685	o, s, d, m	gr	jn-nv	ja-dc	Polb.9,11,21,52,Korn.256,397,400,402,Man.27,35,61,62,63,65,68,Wong.68,San.136,Maxw.B58,60,137,158,165,192	g,s	mt,sk, so,ck, km,dp,nk,dg,dh,st,ppl

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Leucobryum candidum (P. Beauv.) Wils.	ac,cuc, hor	pd	epi	3	egf	1,030-1,295	s,m	gr	?	ja-dc	San.217,231	g	dp
Leucobryum juniperoideum (Brid.) C. Muell.	ac,cuc,inc/hor	pd	epi	3	egf	1,685	s,m	gr	ag-dc	ja-dc	San.103	g,s	dp
Leucobryum microleucophanoides Dix. *ex* A. Johnson	ac,cuc,inc/hor	pd	cor,ram	3	egf	1,300	s,m	gr	sp-dc	ja-dc	Maxw.B129	g,s	dn
Ochrobryum kurzianum Hampe	ac,cuc, mit,ere	pd	cor, ram	3	dof,mxf, egf	30-1,325	o,d,s,m	gr,ss	jl-dc	ja-dc	Polb.6,12,22,59, Prin.4,Maxw.B,136,184	g,s	mt,mk,km
Octoblepharum albidum Hedw.	ac,cuc, ere	pd	epl, cor, ram, lig	4	dof,mxf, def	30-1,685	o, s, d, m	gr	jn-nv	ja-dc	Polb.13,23,60, Prom.25, Prin.32,68,Korn.121,389, 390,Man.35,44,49,61,65, Wong.69,San.137,145,272Maxw.B48,109,135,188 ,Tips.12,Teps.12	g,s	mt,sk, so,ck, km,dp,ak, mk,kbk,ppl
Calymperaceae *Calymperes afzelii* Sw.	ac	a	ter, epl, cor	2	mxf	730	s,m	gr	?	my-dc	Prin.2,20,58, Prom.26	g,gm	mt
Calymperes moluccense Schwaegr.	ac	pd	cor	3	egf (swamp)	75	o,m	ls	?	ja-dc	Maxw.B154	g	kk
Calymperes palisotii Schwaegr.	ac	pd	cor	3	mxf	830	o,s	gr	?	ja-dc	Man. 70,130	g,gm	so
Syrrhopodon armatus Mitt.	ac	pd	cor,ram	2	mxf	850	o,s	gr	?	ja-dc	Man.48	g,gm	so
Syrrhopodon gardneri (Hook.) Schwaegr.	ac	pd	cor	3	egf	1,325-1,685	s,m	gr	?	ja-dc	Wong.63,San.145	g	km,dp
Syrrhopodon tjibodensis Fleisch.	ac	pd	epi	3	egf	1,685	s,m	gr	?	ja-dc	San.136	g	dp

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Syrrhopodon spiculosus var. *patens* (Dix.) A. Eddy	ac	pd	ram	3	egf	2,500	s,m	gr	?	ja-dc	San054/1	g	ak
Syrrhopodon subconfertus Broth.	ac	pd	cor	3	def	1,600	o	gr	?	ja-dc	Korn.394,395	g	sk
Pottiaceae *Barbula consanguinea* (Thwaites & Mitt.) Jaeg.	ac,cuc, ere	a	ter	3	mxf	730	o, m	gr	jn-oc	ap-dc	Prin.53, Polb.27,65,80	g,s	mt
Barbula javanica Dozy & Molk.	ac,cuc, ere	pd	ept	3	egf	1,685	s,m	gr	sp-oc	ja-dc	San.117	g,s	dp
Barbula pseudo-ehrenbergii Fleisch.	ac,cuc, ere	pd	ept	3	mxf	660	s,m	gr	sp-dc	ja-dc	San.205	g,s	dp
Hyophila involuta (Hook.) Jaeg.	ac,cuc, ere	pd	ter, epl	5	mxf,egf	730-1,685	o, s, d, m	gr	jn-oc	ja-dc	Prin.11,14,78,80,87,92 Polb.10,20,32,72, Prom..6,19,58,Man.59,18 3,184,Korn.185,220,257, Maxw.B 8,149,156,169	g,s	mt,so, ck,dp,dt, pn
Hyophila rosea R.S. Williams	ac	pd	ter, epl	3	mxf	730	s, m	gr	?	ja-dc	Prin.74,93	g	mt
Oxystegus sp.	ac	pd	epi	3	egf	1,585	s,m	gr	?	ja-dc	San.141	g	dp
Pseudosymblepharis angustata (Mitt.) Chen	ac,cuc, ere	pd	epi	3	egf	1,685	s,m	gr	oc-dc	ja-dc	San.110	g,s	dp
Trichostomum brachydontium (Brunch.) *ex* C. Muell.	ac,cuc, ere	pd	ept	3	egf	1,685	s,m	gr	sp-nv	ja-dc	San.83	g,s	dp
Trichostomum sp.	ac	pd	ter, epl, cor	3	mxf,def	730-1,600	o, s, d, m	gr	?	ja-dc	Prin.1,5,6,13,14,15, Korn.396	g	mt,sk

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Weissia platystegia (Dix.) Eddy	ac,cuc, im	pd	ter,epl	2	mxf	850	o,d	gr	ag-dc	ja-dc	Man.276	g,s	so
Funariaceae *Entostodon* sp.	ac,cuc, ere	pd	ter	3	mxf,egf, sg	1,550-1,595	s,m	ls	jl-nv	ja-dc	Allen23,Schw.1	g,s	cd,akh
Funaria hygrometrica Hedw.	ac,cuc, hor	pd	ter	3	dof,mxf, egf	350-1,825	o,d,s,m	gr	sp-dc	ja-dc	Man.40,San.123,280,346 ,357,Maxw.B5170,79,10 4,111,144, 191	g,s	so,dp,nk, js,cd,sl,bd, ppl
Physcomitrium pyriforme (Hedw.) Hampe.	ac,cuc, ere	pd	ter	3	urb	330	o,m	gr	jl-dc	ja-dc	Prin.142,Maxw.B90	g,s	cmu,bsk
Splachnaceae *Tayloria indica* Mitt.	ac,mit, ere	pd	epl,ter	3	mxf,egf	1,225-1,500	o,s,m	ls,gr	ag-dc	ja-dc	Allen21,Maxw.B98	g,s	cd,dk
Bryaceae *Brachymenium acuminatum* Harv.	ac	pd	ter	3	egf	1,250	s,m	gr	?	ja-dc	San.059	g	ml
Brachymenium nepalense Hook.	ac,cuc, ere	pd	epi,epl, ter	3	egf	1,500-1,685	s,m	gr	jl-nv	ja-dc	San.101,239,Char.55,Ma xw.B 6,49,67,142,166	g,s	dp,dt,ml,pp l
Brachymenium ochianum Gang.	ac,cuc, ere	pd	epi,cor, lig	3	mxf,egf	1685-1,825	s,m	gr	?	ja-dc	Wong.188,Maxw.B120	g	dp,kj
Brachymenium systylium (C. Muell.) Jaeg.	ac,cuc, ere	pd	ter	3	egf	1,675	s,m	gr	ag-dc	ja-dc	Palee27	g,s	dp
Brachymenium sp.	ac,cuc, ere	pd	ter	3	egf	1,500	s,m	ls	sp-dc	ja-dc	Maxw.B168	g,s	dt
Bryum argenteum Hedw.	ac,cuc, pen	pd	ept	3	egf	1,685	s,m	gr	jl-dc	ja-dc	San.121	g,s	dp
Bryum ausstrale Hampe.	ac,cuc, pen	pd	ept	3	gra	1,800	o,c	ls	ag-dc	ja-dc	Allen87	g,s	cd
Bryum billardieri Schwaegr.	ac,cuc, pen	pd	epi	3	egf	1,685	s,m	gr	jl-dc	ja-dc	San.96	g,s	dp

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Bryum capillare L. *ex* Hedw.	ac,cuc, hor	pd	epi	3	egf	1,685	s,m	gr	ag-dc	ja-dc	San.219	g,s	dp
Bryum coronatum Schwaegr.	ac,cuc, ere,pen	pd	ter, epl	4	urb,dof, mxf,egf	350-2,500	o, m,d, h	gr	jl-nv	ja-dc	Prin.123,Polb.1,18,43,71 ,Prom.23,29Man.114,187 ,214,246,San.199,348,Pa le1101,Maxw.B56,102,3 29,Watt.11,Tunr.3,Makt. 5	g,s	mt,so,mk,h k,wk,cd,ak, cmu,wh,pn g
Bryum russulum Broth.	ac	pd	ter	3	egf	1,685	s,m	gr	?	ja-dc	Prin.141	g,bf	dp
Bryum sandei Dozy & Molk.	ac,culc, inc/hor	pd	ter	3	mxf,egf	1,800	s,m	gr	ag-dc	ja-dc	Allen89	g,s	cd
Mniobryum cf. *ludwigii* (Schwaegr.) Loesk.	ac	pd	ter	2	da,def	1,400	o,m	gr	?	ja-dc	Prin.148	g,gm	ppl
Pohlia sp.	ac,cuc, inc	pd	ept	3	dof,mxf	680	o,d	gr	?	ja-dc	San.347	g	dp
Rhodobryum giganteum (Schwaegr.) Par.	ac,cuc, hor/pen	pd	epi, ter,cor	3	egf	1,320-1,685	s,m	gr	ag-dc	ja-dc	San.142,143,Maxw.B65, 99,147	g,s	dp,pn
Mniaceae *Plagiomnium maximowiezii* (Lindb.) T. Kop.	ac,cuc, hor/pen	pd	epi	3	egf	1,685	s,m	gr	jl-dc	ja-dc	San.154,155	g,s	dp
Plagiomnium succulentum (Mitt.) T.J. Kop.	ac,cuc, hor	pe	emr,sub, ter, epl, cor, ram	4	mxf,egf	730-1,375	s,m,c	gr	ag-dc	ja-dc	Prin.111, Prom.2,Korn.97,176,194, Maxw.8	g,s	mt,ck,dt
Plagiomnium rhynchophorum (Hook.) T.J. Kop.	ac,cuc, hor/pen	pd	ter,epl	3	egf	1,355	s,m	gr	oc-dc	ja-dc	Korn.9	g,s	ck
Rhizogoniaceae *Rhizogonium spiniforme* (Hedw.) Bruch.	ac,mit, inc/hor	pd	ter,epl	4	egf	725-1,319	o,s,m	gr,ss	jn-dc	ja-dc	Wong.308,Maxw.B123, 143,180	g,s	pk,ky

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Bartramiaceae *Philonotis secunda* (Dozy & Molk.) Bosch & Sande Lac.	ac	ped	rhe,ter, epl	4	mxf	730	o,s,m	gr	?	ja-dc	Prin.10,23,57	g	mt
Philonotis speciosa (Griff.) Mitt.	ac	pd	ter	3	dof	350	o,m	gr	?	ja-dc	Makt.2		wh
Philonotis aff. *thaitesii* Mitt.	ac,cuc, ere	pd	ter	3	da	1,700	o,d	gr	jl-nv	ja-dc	Schw.5	g,s	di
Philonotis sp.	ac,ere	pd	ept	3	mxf,egf	670-1,250	o,s,m	gr	?	ja-dc	San.10,208	g	dp
Erpodiaceae *Erpodium biseriatum* (Austin) Austin	pl	pd	cor,ram	3	mxf	730	s,m	gr	jl-nv	ja-dc	Prin.67	g,s	mt
Erpodium mangiferea C. Muell.	pl	pd	cor,ram	4	urb,da	330	o,d,m,	gr	jn-oc	ja-dc	Prin.139	g,s	cmu
Othotrichaceae *Groutiella tomentosa* (Hornsch.) Wijk. & Marg.	ac,mit, ere	pd	cor	3	egf	1,325	s,m	gr	ag-dc	ja-dc	Wong.73	g,s	km
Macromitrium densum Mitt.	ac,mit, ere	pd	cor,ram	3	mxf	870	o,d	gr	oc-dc	ja-dc	Man.67,69	g,s	so
Macromitrium nepalense(Hook. & Grev.) Schwaegr.	ac,mit, ere	pd	cor	3	egf	1,325-1,675	s,m	gr	jl-dc	ja-dc	Wong.74,Palee27	g,s	km,dp
Macromitrium turgidum Dixon	ac,mit, ere	pd	cor	4	def	1,520-1,600	o,s,m	gr	jn-nv	ja-dc	Korn.341-350,San.273,Maxw.148	g,s	sk,dp,pn
Macromitrium zollingeri Mitt. *ex* Dozy & Molk.	ac	pd	cor,ram	3	dof	30	o,m	ss	?	?	Maxw. 2,B179	g	mk
Macromitrium sp.	ac,mit, ere	pd	cor,ram	3	egf	1,350	s,m	gr	ag-dc	ja-dc	Maxw.B92,94	g,s	dk

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Zygodon novoguinensis Bartr.	ac,mit, ere	pd	ept	3	dof,mxf	670	o,m	gr	sp-dc	ja-dc	San.207	g,s	dp
Rachittheciaceae *Rachitthecium perpusillum* (Thwaites & Mitt.) Broth.	ac,mit, ere	pd	cor,ram	3	egf	1,650	s,m	gr	sp-dc	ja-dc	Maxw.B71	g,s	dp
Racopilaceae *Racopilum cuspidigerum* (Schwaegr.) Aongstr.	pl,cuc, ere	pd	cor	3	def,egf	1600-1,685	o,s,m	gr	sp-dc	ja-dc	Korn.391,393,San.110,226,242	g,s	sk,dp
Racopilum orthocarcarpum Wils. *ex* Mitt.	pl,cuc, hor	pd	cor,ram	3	mxf,egf	730-1,658	s,m	gr	sp-dc	ja-dc	Prin.62,San.100,109,182, 187, 194,196,Maxw.68,160,164	g,s	mt,dp,dt,dn
Racopilum sp.	pl	pd	cor	3	egf	775	s,m	ss	?	ja-dc	Maxw.B176	g	ky
Hedwigiaceae *Bryowijkia ambigua* (Hook.) Nog.	pl,im	pd	epi	3	egf	1,685	s,m	gr	jl-dc	ja-dc	San.157	g,s	dp
Trachypodaceae *Trachypodopsis serrulata* (P. Beauv.) Fleisch. var. *crispatula* (Hook.) Zanten	pl	pd	epl,cor	3	egf	1,355-2,500	s,m	gr	?	ja-dc	San.057/1	g	ak
Trachypus bicolor Reinw. & Hornsch.	pl,cuc, ere	pd	epi	3	egf	1,685	s,m	gr	oc-dc	ja-dc	San.98	g,s	dp
Myuriaceae *Myurium rufescens* (Reinw. & Hornsch.) Fleisch.	pl,cuc, ere	pd	epi	3	egf	1,260-1,685	s,m	gr	ag-dc	ja-dc	San.99,102,055	g,s	dp,ml
Pterobryaceae *Pterobryopsis divergens* (Mitt.) Nog.	pl	pd	epi	4	egf	2,200	s,m	ls	?	ja-dc	Allen157	g	cd

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Oedicladium rufescens (Reinw. & Hornsch.) Mitt.	pl,ere	pd	cor	3	def	1,325-1,600	o	gr	jn-nv	ja-dc	Korn.361,362,377, Wong.72	g,s	sk,km
Symphysodontella borii Dix.	pl,cuc, ere	pd	cor,ram	3	egf	1,325	s,m	gr	jl-dc	ja-dc	Maxw.B138	g,s	dp,km
Meteoriaceae *Aerobryidium filamentosum* (Hook.) Fleisch.	pl,cuc/ mit,hor	pd	epi	3	egf	1,685	s,m	gr	sp-dc	ja-dc	San.95,106,131,139,149	g,s	dp
Aerobryopsis longissima (Dozy & Molk.) Fleisch.	pl	pd	cor	3	egf	1,325	s,m	gr	?	ja-dc	Wong.70	g	km
Floribundaria sparsa (Mitt.) Broth. var. *sparsa*	pl,cuc, ere	pd	cor	3	egf	1325-2,685	s,m	gr	sp-dc	ja-dc	k71,San.129/153	g,s	km,dp
Floribundaria walkeri (Ren. *et* Chard) Broth.	pl	pd	cor	3	def	1,600	o	gr	?	jn-sp	Korn.331,332	g	sk
Meteorium miquelianum (C. Muell.) Fleisch. *ex* Broth. subsp. *miquelianum*	pl,cuc, inc	pd	cor	3	def	1,600	o	gr	jn-nv	ja-dc	Korn.351-360,376	g,s	sk
Meteoriopsis reclinata (C. Muell.) Fleisch.	pl,cuc, ere	pd	ept	3	egf	1,685	s,m	gr	sp-dc	ja-dc	San.106,147,159	g,s	dp
Meteoriopsis squarrosa (Hook.) Fleisch. var. *longicuspis* Nog.	pl	pd	cor	3	def,egf	1,425-1,600	o	gr	?	ja-dc	Korn.407, Maxw. 1	g	sk,dt
Papillaria feae Fleisch.	pl	pd	cor	3	def	1,600	o	gr	?	ja-dc	Korn.363-367,379	g	sk
Papillaria fuscenscens (Hook.) Jaeg.	pl,cuc, ere	pd	epi	3	egf	1,685	s,m	gr	ag-dc	ja-dc	San.92,94,104,134	g,s	dp

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Papillaria semitorta (C. Muell.) Jaeg.	pl	pd	cor	3	egf	1,425	s,m	gr	?	ja-dc	Maxw. 3	g	dt
Neckeraceae *Calyptothecium himantocladioides* Bartrum	pl	pd	cor	3	egf	1,350-1,425	s,m	gr	?	ja-dc	Prin. 146, Maxw.173(9)	g	km,dt
Homaliodendron microdendron (Mont.) Fleisch.	pl	pd	epl	3	mxf,egf	730-1,360	s,m	gr	?	ja-dc	Prom.5,10,Korn.92,93,183	g	mt
Homaliodendron obtusatum (Mitt.) Gang.	pl	pd	cor,ram	3	mxf	730	s,m	gr	?	ja-dc	Prom.35,Maxw.B194	g	mt
Neckera himalayana Mitt.	pl	pd	ram	3	egf	2,500	s,m	gr	?	ja-dc	San.0515	g	ak
Neckeropsis exerta (Hook. *ex* Schwaegr.) Broth.	pl,cuc/ mit,ere	pd	cor,ram	2	mxf	730	s,m	gr	sp-dc	ja-dc	Prin.29,Prom.33	g,s	mt
Pinnatella alopecuroides (Hook.) Fleisch.	pl	pd	cor	3	def	1,600	o	gr	?	ja-dc	Korn.368,369,381	g	sk
Hookeriaceae *Actinodontium rhaphidostegium* (C. Muell.) Bosch & Lac.	pl,mit, inc/ hor	pd	epi	3	egf	1,685	s,m	gr	sp-dc	ja-dc	San.146	g,s	dp
Hookeriopsis utacamundiana (Mont.) Broth.	pl,mit, hor	pd	cor,lig	2	egf	1,350-2,500	s,m	gr	ag-nv	ja-dc	Korn.166,Maxw.B66,100 ,San.057/2	g,s	ck,dp,ak
Symphyodontaceae *Symphyodon asper* (Mitt.) Jaeg.	pl,cuc, ere	pd	cor,ram	2	egf	2,500	s,m	gr	jl-oc	ja-dc	San.056	g,s	kp
Hypopterygiaceae *Cyathophorella adiantum* (Griff.) Fleisch.	pl	pd	epi,cor	3	egf	1,200-1,685	s,m	gr	?	ja-dc	San.105,151,4301,Maxw .B114,Wong.& Korn.1	g, bf	dp,js
Cyathophorella hookeriana (Griff.) Fleisch.	pl	pd	cor,ram	3	egf	2,552	s,m	gr	?	ja-dc	Wong.708	g	ak

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Hypopterygium flavolimbatum C. Muell.	pl	pd	cor	2	egf	1,375	s,m	gr	?	ja-dc	Maxw. 4	g	dt
Hypopterygium tenellum C. Muell.	pl	pd	epl,cor	3	egf	1,360	s,m	gr	?	ja-dc	Korn.15,94,188	g	ck
Leskeaceae *Claopodium prionophyllum* (C. Muell.) Broth.	pl	pd	epl	4	mxf	730	s,m	gr	?	ja-dc	Korn.6,25,96,98,161Prom.15	g	ck,mt
Thuidiaceae *Thuidium glaucinum* (Mitt.) Bosch & Lac.	pl,cuc, hor	pd	epi	3	egf	1,685	s,m	gr	sp-dc	ja-dc	San.110	g,s	dp
Thuidium plumulosum (Dozy & Molk.) Dozy & Molk.	pl,cuc, hor	pe	cor,ram	4	mxf,egf	50-1,355	s,m	gr	ag-dc	ja-dc	Prom.rin6,43,54,72, Polb.7,25,54,75, Prom.18,51,52, Man.222,322,325, 340,341,Korn.133,164, San.204,Maxw.B75,153	g,s	mt,so, dp,kk,ms
Thuidium venustulum Bosch	pl,cuc, ere	pd	epi	3	egf	1,685	s,m	gr	oc-dc	ja-dc	San.195	g,s	dp
Brachytheciaceae *Brachythecium buchananii* (Hook.) Jaeg.	pl,inc	pd	ept	3	egf	1,520	s,m	gr	?	ja-dc	San.275	g	dp
Eurhynchium celebicum (Sande Lac.) Bartram	pl	pd	epl	3	egf	1,355	s,m	gr	ag-nv	ja-dc	Korn.1,11,23,44,99,103, 155	g	ck
Rhynchostegium aff. *psilopodium* Igna. & Hatt.	pl,cuc, inc/hor	pd	lig	3	dof	500	s,m	sh	ag-dc	ja-dc	Maxw.B162	g,s	my
Entodontaceae *Entodon curvatus* (Griff.) Jaeg.	pl,cuc, ere,imc	pd	epl	3	egf	1,685	s,m	gr	jl-dc	ja-dc	San.191,221	g,s	dp
Entodon macrocarpus (Hedw.) Mitt.	pl,cuc, ere	pd	cor	3	egf	1,350	s,m	gr	jl-dc	ja-dc	Maxw.B140,155,187	g,s	dt,dn
Entodon macropodus (Hedw.) C. Muell.	pl,cuc, ere	pd	epi,cor, ram	3	mxf,egf	1,050-1,800	s,m	ls,gr	jl-dc	ja-dc	Allen176,Maxw.B119	g,s	cd,kj

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Entodon plicatus C. Muell	pl,cuc, ere	pd	epi	3	egf	1,685	s,m	gr	sp-dc	ja-dc	San.144,Maxw.B170	g,s	dp,dt
Entodon sp.	pl,cuc, ere	pd	cor	3	bb/df,egf	900-1,000	s	ls,gr	jl-dc	ja-dc	Maxw.B106,151	g,s	db,js
Erythrodontium julaceum (Schwaegr.) Par.	pl,cuc, ere	pd	cor,ram, lig	4	dof,mxf, egf	730-850	o,m,d	gr	jl-nv	ja-dc	Prin.73,88,Maxw.B77,141,182	g,s	mt,td,ky,ms
Trachyphyllum inflexum (Harv.) Gepp.	pl,cuc, inc	pd	cor	3	dof,mxf	570	o,s,m	ls	ag-dc	ja-dc	Allen65	g,s	cd
Trachyphyllum sp.	pl	pd	cor,ram	2	mxf	730	s,m	gr	?	ja-dc	Prin.37,52,56	g	mt
Plagiotheciaceae *Plagiothecium neckeroideum* B. S. G.	pl	pd	ram	3	egf	2,500	s,m	gr	?	ja-dc	San.058	g	ak
Stereophyllum decorum (Mitt.) Wijk. & Margad.	pl,ere	pd	epl	2	mxf	730	o,d	gr	sp-dc	ja-dc	Prin.3	g,s	mt
Stereophyllum sp.	pl,cuc, inc	pd	cor	3	egf	1,000	s,m	gr	sp-dc	ja-dc	Maxw.B163	g,s	dt
Sematophyllaceae *Acroporium diminutum* (Brid.) Fleisch.	pl,cuc, inc	pd	epi	3	egf	1,685	s,m	gr	oc-dc	ja-dc	San.113,124	g,s	dp
Chionostomum rostratum (Griff.) C. Muell.	pl	pd	cor	3	egf	1,325	s,m	gr	?	ja-dc	Wong.75	g	km
Clastobryella merrilli (Broth.) Fleisch.	pl	pd	cor	3	egf	1,325	s,m	gr	?	ja-dc	Wong.77	g	km
Gammiella pterogonoides (Griff.) Broth.	pl	pd	cor	3	egf	1,325	s,m	gr	?	ja-dc	Wong.78	g	km

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Species	Habit	Aped	Life-mode	Abun-dance	Habitat	Eleva1tion (m)	Micro-habitat	Bed-rock	Sporophyte month	Gametophyte month	Collector number	Stage	Location
Gammiella tonkinense (Broth. & Par.) B. C. Tan	pl	pd	cor	3	def	1,325-1,600	o,s,m	gr	?	ja-dc	Korn.405,406,Wong.79	g	sk,km
Heterophyllium affine (Hook.) Fleisch.	pl,cuc, inc	pd	cor,ram	3	dof	850	o,m	gr	jl-nv	ja-dc	Maxw.B64	g,s	so
Pseudotrismegistria undulata (Broth. & Yas.) Akiyama & Tsubota	pl	pd	cor	4	egf	2,556	s,m	gr	?	ja-dc	Wong.742,San.0511/1	g	ak
Radulina hamata (Dozy & Molk.) W. R. Buck & B. C. Tan	pl,cuc, inc	pd	cor	3	egf	1,360	s,m	gr	sp-dc	ja-dc	Korn.8,55	g,s	ck
Taxithelium nepalense (Schweagr.) Broth.	pl, cuc, inc	pd	epl, cor,lig	3	mxf,bb/ df	730	s,m,o,d	gr	jn-nv	ja-dc	Polb.8,38,62,Maxw.B17 3 (5),177	g,s	mt,mk,dt
Taxithelium oblongifolium (Sull. & Lesq.) Z. Iwats.	pl,cuc, hor	pd	cor,ram	3	mxf	730-850	s,m	gr	jl-nv	ja-dc	Prin.51,Prom.48,Man.12 4,127,241,246,300,311	g,s	mt,so
Taxithelium pavulum (Broth. & Par.) Broth.	pl,cuc, pen	pd	epi	3	egf	1,685	s,m	gr	oc-dc	ja-dc	Prom.38	g,s	dp
Trichosteleum bistrummosum (C. Muell.) Jaeg.	pl,cuc, inc	pd	cor,ram	3	mxf	730	s,m	gr	jl-nv	ja-dc	San.246	g,s	mt
Trichosteleum stigmosum Mitt.	pl,cuc, inc	pd	cor	3	egf	15	s,m	sl	?	ja-dc	Maxw.B189	g,s	kb
Sematophyllum phoenicum (C. Muell.) Fleisch.	pl	pd	cor	3	egf	1,325	s,m	gr	?	ja-dc	Wong.80	g	km
Sematophyllum subhumile (C. Muell.) Fleisch.	pl	pd	epl,cor, ram	3	mxf,egf	850-1,685	o,d,s,m	gr	sp-dc	ja-dc	Man.124,241,246,300,31 1, Korn.217,219,San.158	g,s	so,ck, dp
Wijkia sercularis (Mitt.) Crum.	pl	pd	cor	3	egf	1,325	s,m	gr	?	ja-dc	Wong.81	g	km
Hypnaceae *Ectropothecium dealbatum* (Reinw. & Hornsch.) Jaeg.	pl,cuc, hor	pd	ept,lig	3	mxf,egf	800-1,010	s,m	gr	jl-dc	ja-dc	San.209, Maxw.B 150	g,s	dp,db

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Ectropothecium monumentorum (Dub.) Jaeg.	pl,cuc, inc/hor	pd	cor,epl	3	def	1600-1,685	o,s,m	gr	ag-dc	ja-dc	Korn.327,328,329,330,383, San.122, Maxw.B185	g,s	sk,dp
Hypnum plumaeforme Wilson	pl,cuc, inc	pd	epl	3	dof,egf	525-1360	o,d,s,m	gr	sp-dc	ja-dc	Korn.230	g,s	ck,na
Hypnum sp.	pl,cuc, hor,pen	pd	cor	3	egf	755	s,m	ss	sp-dc	ja-dc	Maxw. B176	g,s	ky
Isopterygium albescens (Hook.) Jaeg. var. *smallii* (Sull. & Lesq.) Z. Iwats.	pl	pd	cor	3	egf	1,325	s,m	gr	?	ja-dc	Wong.67	g	km
Isopterygium bancanum (Sande Lac.) Jaeg.	pl,cuc, pen	pd	cor	3	egf	1,360	s,m	gr	ag-nv	ja-dc	Korn.237	g,s	ck
Isopterygium distichaceum (Mitt.) Jaeg.	pl	pe	epl	3	mxf	730	o,m	gr	?	ja-dc	Polb.5,48,76, Makt.9	g	mt
Isopterygium lignicola (Mitt.) Jaeg.	pl,cuc, hor	pd	epi	3	dof	350	o,d	gr	ag-dc	ja-dc	San.198	g,s	dp
Isopterygium minutirameum (C. Muell.) Jaeg.	pl,cuc, inc	pd	ept	3	mxf,egf	1,580	o,m	ls	ag-dc	ja-dc	Allen57	s	cd
Isopterygium serrulatum Fleisch.	pl	pd	ter, epl	3	mxf	730	s,m	gr	?	ja-dc	Prin.16		mt
Isopterygium tenerum (Schwaegr.) Mitt.	pl	pe	cor,ram	2	mxf	730	s,m	gr	?	ja-dc	Prom.17	g,bf	mt
Isopterygium sp.	pl,cuc, inc	pd	cor,lig	4	egf	800-1,125	s,m	ss,gr	ag-dc	ja-dc	Maxw.B84,181	g,s	ky,dp
Vesicularia dubyana(C. Muell) Broth.	pl	pd	ter	3	da,def	1,400	o,m	gr	?	ja-dc	Prin. 149	g	ppl

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Vesicularia montagnei (Schimp.) Broth.	pl	pd	epl	3	egf	1,355	s,m	gr	?	ja-dc	Korn.21,23	g	ck
Hylocomiaceae *Macrothamnium macrocarpum* (Reinw. & Honsch.) Fleisch.	pl,cuc, hor	pd	cor,ram	3	mxf,egf	730-1,685	s,m	gr	ag-nv	ja-dc	Prin.90,San.120, 128,148,223,225,245,247	g,s	mt,dp
Polytrichaceae *Atrichum yakusiamensis* (Hor.) Miz.	ac	pd	ter,epl	3	mxf	730	s,m	gr	?	ja-dc	Prin.25,30	g	mt
Atrichum crispum (Jam.) Sull. & Lesq.	ac,cuc, ere	pd	ter	3	def,egf	1,150	o,m	gr	ag-dc	ja-dc	Maxw.B96	g,s	msl
Pogonatum neesii (C. Muell.) Dozy	ac,mit, ere	pd	ter	4	mxf,egf, da	500-1,825	o,d,h	gr	ag-nv	ja-dc	Prin.31, Polb.24,53,61,83,San.114,190,200,215,951Petr.460,Maxw.B47,55,57,86,95,108,115,118,127,134,139,145,147157,Char.56,Schw.2	g,s	mt,dp,ma,ppl,bhf,dh,dn,js,sl,msl, dlnp,akh,ck,rs,ak
Pogonatum proliferum (Griff.) Mitt.	ac,mit, ere	pd	ter	4	egf	2,500	s,m	gr	jl-dc	ja-dc	Wong.740	g,s	kp
Pogonatum c.f. *subtortile* (C. Muell.) Jaeg.	ac,mit, ere/inc	pd	ept	3	egf	1,685	s,m	gr	sp-dc	ja-dc	San.116	g,s	dp
Hepaticopsida Lepidoziaceae *Bazzania javanica* (Sde. & Lac.) Schiff.	sl	pe	cor	3	egf	2,556	s,m	gr	?	ja-dc	Wong.725	g	ak
Bazzania tridens (Reinw., Blume & Nees) Trevis	sl	a	ter, cor	4	mxf,def	730-1,600	o,s,m	gr	?	ja-dc	Prin.122, Polb.28,69, Korn.409,Man.3,311	g	mt,sk, so
Telaranea sp.	sl	pe	epl,cor	3	egf	2,500	s,m	gr	?	ja-dc	Wong.789	g	kp
Trichocalea tomentella (Eheh.) Dum.	sl	pe	ram	4	egf	2,500	s,m	gr	?	ja-dc	San.052	g	ak

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Herbataceae *Herbatus* sp.	sl	pe	cor	3	egf	2,500	s,m	gr	?	ja-dc	San.051	g	ak
Calypogeiaceae *Calypogeia arguta* Nees & Mont.	sl,ere	a	ter	3	mxf	850	s,m	gr	dc-ja	ja-dc	Man.42,331,334,338	g,s	so
Geocalycaceae *Heteroscyphus argutus* (Nees) Schiff.	sl	a	ter,epl	3	mxf,egf	730-1,360	s,m	gr	?	jn-oc	Prin.96,Prom.37,Man.22, 66,81,128, 202, Korn.50,52	g	mt,so,ck
Heteroscyphus coalitus (Hook.) Schiff.	sl	a	epl	2	mxf	730-830	s,m	gr	?	jn-oc	Prom.11,Man.23,34,71,78,91,156	g	mt,so
Heteroscyphus zollongeri (Gott.) Schiff.	sl	a	ter,epl	3	mxf,egf	730-1,360	s,m	gr	?	ja-dc	Prin.17,28,35,39,95,Polb.14,33,77,Prom.13,47,50, 67,Man.10,40,128, 251,313,Korn.3,11,22,30,32	g	mt,so,ck
Lophocolea morobaena Piippo.	sl,ere	a	ter,epl	4	mxf	730-830	s,m	gr	jl-oc	my-dc	Prin.18,97,Prom.12,27,Man.19,85,90, 115,126,225,Korn.157	g,s	mt,so, ck
Lophocolea minor Nees	sl	a	ter	2	mxf	730-830	s,m	gr	?	my-sp	Prin.98, Polb.25,54, Prom.73,Man.23,256	g	mt,so
Jungermanniaceae *Jungermannia tetragona* Lindenb.	sl,ere	a	ter	4	mxf	730	s,m	gr	my-oc	my-dc	Prin.34,50,101	g,s	mt
Jungermannia truncata Nees	sl,ere	a	ter	4	mxf	730-850	s,m	gr	my-oc	my-dc	Prin.102,Polb.49,Man.97,103,131, 183,196,227,279,288	g,s	mt,so
Notoscyphus paroicus Schiff.	sl,ere	a	ter	5	mxf	730	o,m	gr	jl-nv	ap-dc	Polb.36	g,s	mt
Plagiochilaceae *Plagiochila* sp.	sl	a	epl,cor	3	mxf	730-830	s,m	gr	?	my-oc	Prin.107,Man.58	g	mt,so
Plagiochila junghuhniana Sande Lac.	sl	pe	epl	3	mxf	730	s,m	gr	?	ja-dc	Prom.14,30,53,54,55,65	g	mt
Plagiochila parvifolia Lindenb.	sl	pd	epl,cor	3	def	1,360-1,600	o	gr	?	ja-dc	Korn.81,370,371,384	g	sk,ck

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Plagiochila sciophila Nees	sl	pd	epl,cor	3	egf	1,355	s,m	gr	?	ja-dc	Korn.11,26	g	ck
Plagiochila semidecurrens (Lehm. & Lindenb.) Lindenb.	sl	pd	cor	3	def	1,600	o	gr	?	ja-dc	Korn.408	g	sk
Cephaloziaceae *Cephalozia siamensis* N. Kitag.	sl	a	ter	1	mxf	730-850	s,m	gr	?	my-sp	Prin.33,100,Man.191,198	g	mt,so
Cephaloziellaceae *Cephaloziella stephanii* Schiff. *ex* Douin	sl,ere	a	ter	2	mxf	850	s,m	gr	oc-dc	my-dc	Man.156,171,,272	g,s	so
Cylindrocolea tagawae (N. Kitag.) R. M. Schust.	sl	a	ter	2	mxf	850	s,m	gr	?	my-dc	Man.32,159,183	g	so
Porellaceae *Porella acutifolia* (Lehm. & Lindenb.) Trevis	sl	a	epl	2	mxf	730	s,m	gr	?	my-nv	Prin.106	g	mt
Porella acutifolia (Lehm. & Lindenb.) Trev. ssp. *latior* Hatt.	sl	a	epl	3	mxf,egf	730-1,360	s,m	gr	?	my-dc	Prom.68,Man.113,122,Korn.83	g	mt,so,ck
Porella acutifolia (Lehm. & Lindenb.) Trev. ssp. *tosana* (Stephani) S. Hatt.	sl	a	epl	2	mxf	850	s,m	gr	?	ja-dc	Man.114,123,258	g	so
Porella plumosa (Mitt.) Hatt.	sl	a	epl	2	mxf	730	s,m	gr	?	my-dc	Prom.69	g	mt
Frullaniaceae *Frullania ericoides* (Nee *ex* Mart.) Mont.	sl	pd	epl,cor, ram	3	mxf	730-850	o,d	gr	?	ja-dc	Polb.46,74,Man.49,224	g	mt,so
Frullania galeta (Reinw., Nees, & Bl.) Dumort.	sl	pd	cor	3	egf	1,400	s,m	gr	?	ja-dc	Korn.54,106,174,224	g	ck

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Frullania gemmulosa S. Hatt. & Thaithong	sl	pd	cor	3	egf	1,400	s,m	gr	?	ja-dc	Korn.254	g	ck
Frullania muscicola Steph.	sl	pd	cor	3	def	1,355-1,600	o	gr	?	jn-sp	Korn.57,58,79,80,105,120,132	g	sk,ck
Frullania nepalensis (Spruce) Lehm. & Lindenb.	sl	pd	cor	3	def	1,600	o	gr	?	jn-oc	Korn.333-338,378,387	g	sk
Frullania wallichiana Mitt.	sl	pd	cor	3	def	1,600	o	gr	?	jn-sp	Korn.340	g	sk
Frullania meyeniana Lindeb.	sl	pd	cor,ram	3	mxf	850	o,d	gr	?	ja-dc	Man.262,283	g	so
Frullania sp.	sl	pd	cor,ram	3	egf	1,610	s,m	gr	?	ja-dc	Maxw. B74	g	dp
Lejeuneaceae *Acrolejeunea fertilis* (Reinw., Bl., & Nees) Spruce *ex* Steph.	sl	pd	cor,ram	3	mxf	730-890	s,m	gr	?	ja-dc	Prin.1,119,129, Polb.2,19,26,68,Man.16, 43,61,62,99,100,106,205 ,262	g	mt,so
Archilejeunea planiuscula (Mitt.) Steph.	sl,ere	pd	cor,ram	3	mxf	730	s,m	gr	?	ja-dc	Prin.19,124	g,s	mt
Cheilolejeanea intertexta (Lindenb.) Steph.	sl	pd	cor,ram	3	mxf	730-830	s,m	gr	?	ja-dc	Prin.112,133,Man.56,66, 317	g	mt,so
Cheilolejeanea obtusilobula (S. Hatt.) Mitzut.	sl	pd	cor,ram	3	mxf	730	s,m	gr	?	ja-dc	Prin.121,126	g	mt
Cololejeunea yakusimensis (S. Hatt.) S. Hatt.	sl	pd	epl,cor, ram,epp	4	mxf	730	o,s,m,d, h	gr	?	ja-dc	Prin.66, Polb.29,70	g	mt
Cololejeunea lanciloba Steph.	sl	pe	epl,cor, ram	3	mxf,egf	830-1,300	o,d,s,m	gr	?	ja-dc	Man.24,65,78,94,107,155,193,224,287,Korn.141, 149,270,172 ,228	g	so,ck
Cololejeunea spinosa (Horik.) Pande´ & R. N. Misra	sl	pe	epp	2	egf	1,300	s,m	gr	?	ja-dc	Korn.158,172	g	ck

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Lejeunea flava (Sw.) Nees	sl	pd	cor	3	def	1,360-1,600	o	gr	?	ja-dc	Korn.42,63,66,82,106,403,404	g	sk,ck
Lejeunea punctiformis Taylor	sl	pd	cor,ram	3	mxf	850	o,d	gr	?	ja-dc	Man.24,65,78,94,107,155,193,224,287	g	so
Lejeunea cf. obscula Mitt.	sl	a	epl	2	mxf	730	s,m	gr	?	my-dc	Prom.70	g	mt
Lejeunea tuberculosa Steph.	sl	pd	cor	3	mxf	730	s	gr	?	ja-dc	Prin.105,120,125,129,130,132	g	mt
Leptolejeunea elliptica (Lehm. & Lindenb.) Schiff.	sl	a	epp	2	egf	1,355	s,m	gr	?	ja-dc	Korn.151	g	ck
Lopholejeunea ceylanica Steph.	sl	a	epi	3	egf	1,566	s,m	gr	?	ja-dc	Korn.441	g	hs
Lopholejeunea nigricans (Lindenberg) Schiff.	sl	a	epi	3	egf	1,586	s,m	gr	?	ja-dc	Korn.449	g	hs
Mastigolejeunea indica Steph.	sl,ere	pd	epl,cor, ram	3	mxf	730-850	s,m	gr	ag-nv	ja-dc	Prin.116,117,118,131,134, Man.115,154,204,223,327	g,s	mt,so
Mastigolejeunea ligulata (Lehm. & Lindenb.) Schiff.	sl	pd	epi	3	mxf	573	o,d	gr	?	ja-dc	Korn.456	g	pd
Mastigolejeunea repleta (Taylor) Evans.	sl,ere	pd	epl,cor, ram	3	mxf	730	s,m	gr	ag-dc	ja-dc	Prin40,115,127,130, Polb.56,57,86	g,s	mt
Ptycanthus striatus (Lehm. & Lindenb.) Nees	sl	a	epl	2	mxf,egf	730-1,400	s,m	gr	?	my-dc	Prom.71,Korn.78,San.05 11/2	g	mt,ck,ak
Schiffneriolejeunea sp.	sl	pd	epi	3	egf	1,566	s,m	gr	?	ja-dc	Korn.448	g	hs
Spruceanthus polymorphus (Sande Lac.) Verd.	sl	pd	cor,ram	3	mxf,egf	730-1,355	s,m	gr	?	ja-dc	Prom.16,20,32,Korn.133,186	g	mt,ck

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Spruceanthus semirepandus (Nees) Verd.	sl	pd	cor	3	def	1,600	o	gr	?	ja-dc	Korn.372,375,380,398,435-438,444,446	g	sk,hs
Pleuroziaceae *Pleurozia* sp.	sl	pd	cor	4	egf	2,500	s,m	gr	jl-oc	ja-dc	San.0514	g,s	kp
Fossombroniaceae *Fossombronia cristula* Austin	sl,ere	a	ter	3	mxf	730-850	s,m	gr	my-oc	my-oc	Prin.99,Man.112,118,198,201,215	g,s	mt,so
Fossombronia pusilla (L.) Nees	sl,ere	a	ter	4	egf	1,685	s,m	gr	jn-oc	my-dc	Man.081	g,s	dp
Pallaviciniaceae *Pallavicinia lyellii* (Hook.) Carruth.	th	a	epl	2	mxf	730-830	s,m	gr	?	ja-dc	Prom.74,Man.98,137	g	mt,so
Aneuraceae *Aneura* sp.	th	a	cor	3	egf	1,500	s,m	gr	?	ja-dc	Maxw.B59	g	ppl
Aneura pinguis (L.) Dumort.	th	a	ter	3	mxf, da	1,250	s,m	gr	?	ja-dc	QSBG6	g	QSBG
Riccardia bipinnatifida (Colenso) Hewson	th, ere	a	ter,epl	3	mxf	730-810	s,m	gr	?	ap-sp	Prin.114, Prom.7,Man.98,147,159,274,316	g,s	mt,so
Riccardia platyclada Schiff.	th	a	ter	3	mxf	730-810	s,m	gr	sp-dc	my-nv	Prin.94,Man.78,114,143,251	g,s	mt,so
Metzgeriaceae *Metzgeria* sp.	th	a	cor,ram	3	egf	1,685-2,500	s,m	gr	?	ja-dc	San.0510,0512	g	kp,dp
Pelliaceae *Pellia* sp.	th	a	ter	3	egf	1,685	s,m	gr	jl-ag	jn-oc	San.0513	g,s	dp
Aytoniaceae *Asterella blumeana* (Nees) Pande´, K. P. Srivast., & Sultan	th	a	ter,epl	4	mxf,egf	730-1,360	s,m	gr	my-sp	ap-sp	Polb.56,Korn.145,203,214,250,252	g,s	mt,ck
Asterella sp.	th	a	ter	3	dof,mxf, egf	350-1,610	o,m	gr	?	ja-dc	Tunr.10,Char.57,Maxw.B62,63,72,76,87,101,112	g,s	mhy,dp,cd, ck,dp,km, mhf,mm,st
Targioniaceae *Targionia hypophylla* L.	th	a	epl	2,4	egf	1,300-1,375	s,m	gr	?	my-dc	Korn.86,245	g,cs	ck, dt

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Marchantiaceae *Dumortiera hirsuta* (Sw.) Nees	th	a	sub,epl, ter	2	mxf,egf	830-1,360	s,m	gr	?	ja-dc	Man.97,137,179,273,326 ,Korn.18,33,37,72,101,1 91,247,Maxw.B73,107,1 59,175	g	so,ck,dh, ky,js,cd
Marchantia sp.	th	a	ter	3	dof,bb/ df,egf,de f	550-1,457	s,m	gr	?	ja-dc	Maxw.B46,52,85,89,91,9 7,110,116,133	g	ck,dma ,bnh,cd, ppl,sf,dl,st
Cyathodiaceae *Cyathodium* sp.	th	a	ter,epl	4	erb	350	s,m	gr	?	jl-nv	Prin.145	g	cmu
Ricciaceae *Riccia fluitans* L.	th	a	flt,emr, sub, rhe,ter	3	mxf	730	o,s,m,c	gr	jn-dc	ja-dc	Prin.19,113	g,cs	mt
Riccia sp.	th	a	ter	3	dof	75	o,m	ss	?	ja-dc	Maxw.B121,183	g	mk
Anthocerotopsida Anthocerotaceae *Anthoceros subtilis* Steph.	th,ere	a	ter	2	mxf	850	o	gr	ag-nv	ja-dc	Man. 188,227	g,s	so
Anthoceros sp.	th,ere	a	ter	3	bb/df, mxf	600-1,575	s,m	gr	jn-sp	my-dc	Maxw.B61,78,88,103,10 5,113,117,122	g,s	cd,kbk,hk,j s,ck,dsl,bd, st
Phaeoceros laevis (L.) Prosk.	th,ere	a	epl,ter	2	mxf,def	730-1,350	o,m	gr	jl-nv	my-dc	Korn.146,204,206,207,M axw.B152,174	g,s	mt,ck,dh, sl
Phaeoceros laevis (L.) Prosk. subsp. *carolinianus* (Mich.) Prosk.	th, ere	a	ter	3	egf	1,150	o,s,m	ss	ag-dc	my-ja	Maxw.B190	g,s	nk
Phaeoceros sp.	th,ere	a	ter	3	bb/df	975	s,m	gr	ag-oc	my-dc	Palee173	g,s	dk
Dendrocerotaceae *Megaceros fragellaris* (Mitt.) Steph.	th	a	emr,sub	3	mxf	730-810	s,m	gr	?	ja-dc	Prom.42,44,64, Man.96,132,136,326	g	mt,so
Notothyladaceae *Notothylas javanica* (Sande Lac.) Gott.	th,hor	a	ter	3	mxf	850	o,s	gr	sp-nv	my-dc	Man. 189,228	g,s	so
Notothylas orbicularis (Schwein.) Sull.	th,hor	a	ter	2	mxf	850	o,s	gr	ag-nv	my-dc	Man. 190,229	g,s	so
Notothylas sp.	th	a	epl	3	mxf,egf	1,400	o,m	gr	?	ja-dc	Korn.147,151	g	ck

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Meaning of Abbreviations

HABIT: **Gametophyte:** sl stem-like and leaf-like th thallus ac arocarpous pl pleurocarpous

Sprophyte: **calyptra:** cam campanulate cuc cucullate mit mitrate

capsule: ere erect inc inclined hor horizontal pen pendulous im immerged

APED: a annual pe perennial evergreen pd perennial deciduous ped perennial evergreen-deciduous

LIFE_MODE: aqu aquatic ept epiterrestial epi epiphyte epl epilithic

flt floating ter tericolous cor corticolous rup rupicolous cul cultivated

emr emerged lit litter ram ramicolous nat naturalised

sub submerged epp epiphyllous int introduced

rhe rheophyte lig lignicolous

ABUNDANCE: 0 Probably extirpated

1 Down to a few individuals, in danger of extirpation

2 Rare

3 Medium abundance

4 Common, but not abundant

5 Abundant

HABITAT:

dof	deciduous dipterocarp-oak seasonal hardwood forest
bb/df	bamboo+deciduous seasonal forest
do/pine	pine+ deciduous dipterocarp forest
mxf	mixed evergreen+deciduous seasonal hardwood forest
eg/bb	primary evergreen+bamboo seasonal hardwood forest
eg/pine	primary evergreen+pine seasonal hardwood forest
egf	primary evergreen seasonal hardwood forest
def	degraded evergreen forest
da	disturbed areas, roadsides
sg	secondary growth
gra	grassland
be	beaches
agr	agricultural areas
urb	urban

MICROHABITAT: o open s shaded d dry m moist c cool h hot

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BEDROCK: gr granite ls limestone qz quartzite sh shale ss sandstone ms metamorphic sandstone

SPOROPHYTE MONTHS: ja fb mr ap my jn jl ag sp oc nv dc = January - December

GAMETOPHYTE MONTHS: ja fb mr ap my jn jl ag sp oc nv dc = January - December

STAGE: g gametophyte s sporophyte cs cleistocarpous sporophyte gm gemmae bf brood filament

Collectors: **Allen** D. Allen

Char. P. Charoenchai

Korn. S. Kornochalert

Makt. P. Maktrairut

Man. S. Manachit

Maxw. J. F. Maxwell

Pale P. Pale

Petr. O. Petrmitr

Polb. M. Polboonsri

Prin. N. Printarakul

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San. K. Santanachote

Schw. P. Schwendinger

Teps. A. Tepsiriumnouy

Tunr. M. Tunruttanakul

Watt. S. Wongwattanaphaibool

Wong. K. Wongkuna

Collecting Localities

ak Doi Ang Ka, Doi Inthanon National Park, Jawm Tong District, Chiang Mai Province

akh Ang Kahng, Fang District, Chiang Mai Province

bd Bong Duat Hot Spring, Mae Dtang District, Chiang Mai Province

bhf Bahng Hin Fohn, Mae Jam District, Chiang Mai Province

bnh Ban Nawng Hoy (village), Mae Rim District, Chiang Mai Province

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bsk Ban Saen Kum (village), Sahn Bah Dtong District, Chiang Mai Province

cd Doi Chiang Dao National Park and Wildlife Sanctuary, Chiang Dao District, Chiang Mai Province

ck Kuhn Chang Kian village, Doi Sutep-Pui National Park, Muang District, Chiang Mai Province

cmu Chiang Mai University, Muang District, Chiang Mai Province

db Doi Pah Baw, Bahng Mah Pah District, Mae Hawng Sawn Province

dg Doi Giah, Mae Fa Luang District, Chiang Mai Province

dh Doi Bahng Mah Hahn (Akha) village, Mae Fa Luang District, Chiang Rai Province

dhl Doi Hoa Loh, Mae Jam District, Chiang Mai Province and Khun Yuam District, Mae Hawng Sawm Province

di Doi Intanon, Doi Intanon National Park, Jawm Tong District, Chiang Mai Province

dk Doi Khun Dthan National Park, Mae Tah District, Lampoon Province

dl Doi Langka Luang, Kuhn Jae National Park, Chiang Mai Province

dlnp Doi Luang National Park, Wahng Nua District, Lampang Province and Pan District Chiang Rai Province

dma Doi Mawn Angget, Sa Meung District, Chiang Mai Province

dn Doi Mawn Ngaw, Mae Dtang District, Chiang Mai Province

dp Doi-Pui, Doi Sutep-Pui National Park, Muang District, Chiang Mai Province

dsl Doi Sahng Liang, Mae Dtang District, Chiang Mai Province

dt Doi Dtung (Tung), Mae Sai District, Chiang Rai Province

hk Huay Keaw (Gayo) water Falls, Doi Sutep-Pui National Park, Muang District, Chiang Mai Province

hs Doi Hua Sua**,** Doi Inthanon National Park, Jawm Tong District, Chiang Mai Province

js Jae Sawn National Park, Muang Bahn District, Lampang Province

kb Emerald Pool, Klong Tawm District, Krabi Province

kbk Kan Bauk village, Yebyu Township, Tawer District, Tenasserim Division, Myanmar (Burma)

kj Kuhn Jae National Park, Wieng Bah Bao District, Chiang Rai Province

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kk Kao Pra Bahng Kram Wildlife Sanctuary, Klong Tawm District, Krabi Province

km Huay Kawk (Kog) Ma, Doi Sutep-Pui National Park, Muang District, Chiang Mai Province

kp Kew (Giew) Mae Pan (Bahn), Doi Intanon National Park, Jawm Tong District, Chiang Mai Province

ky Kao Yai National Park, Nakorn Ratchasima Province and Nakorn Nayok Province

ma Mae Rah Ah watershed, Om Koi District, Chiang Mai Province

mk Mae Kong river, Sambour District, Kratie Province, Cambodia

ml Doi Mawn Lawng**,** Doi Sutep-Pui National Park, Mae Rim District, Chiang Mai Province

mm Mae Sa Mai, Mae Rim District, Chiang Mai Provice

mt Montatahn Falls, Doi Sutep-Pui National Park, Muang District, Chiang Mai Province

mhf Mae Ha Falls, Hang Dong District, Chiang Mai Province

mhy Mae Hai Ya, Muang District, Chiang Mai Province

ms Mae Sa Falls, Mae Rim District, Chiang Mai Province

msl Doi Mae Sa Long, Mae Chan District, Chiang Mai Province

my Mae Yom National Park, Song District, Prae Province

na Nakai Plateau, Nakai District, Savannaket Province, Laos

nk Nong Khao Klang (Karen) village, Muang District, Mae Hong Sawn Province

nl Nam Dtok Huay Sai Laung Falls, Doi Inthanon National Park, Jawm Tong District, Chiang Mai Province

nn Nam Dtok Ngao Falls, Nam Dtok Ngao National Park, Ranong Province

pd Pha Dang National Park, Chiang Dao District, Chiang Mai Province

php Phu Pan National Park, Sakon Nakorn Province

pk Phu Hin Rong Kla, Phu Hin Rong Kla Naional Park, Pitsanulok Province

pn Pah Ngaem limestone, Mae Wang District, Chiang Mai Province

png Pah Ngeub, Muang District, Chiang Mai Province

pp Phanom Pencha National Park, Krabi Province

ppl Puping Palace, Muang District, Chiang Mai Province

QSBG Queen Srikit's Botanic Garden, Mae Rim District, Chiang Mai Province

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rs Ru See Cave, Muang District, Chiang Mai Province

td Tahm Dahgadan, Hang Dong District, Chiang Mai Province

sf Siripum Falls, Doi Intanon National Park, Jawm Tong District, Chiang Mai Province

sk San Ku (Gu), Doi Sutep-Pui National Park, Muang District, Chiang Mai Province

sl Summit of Doi Lohn, Muang Bahn District, Lampang Province and Mae Awn District, Chiang Mai Province

so Sirindhorn Observatory, Doi Sutep-Pui National Park, Muang District, Chiang Mai Province

st Doi Sutep Temple, Muang District, Chiang Mai Province

wh Wat (temple) Fai Hin, Muang District, Chiang Mai Province

wk Wat (temple) Chang Kian, Muang District, Chiang Mai Province

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Full Paper

Nondestructive measurement of sugar content of apple using hyperspectral imaging technique

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Abstract: Hyperspectral imaging technique is an upcoming and promising field of research for non-destructive quality assessment of agricultural and food products. It has a greater advantage of combining spatial imaging and spectral measurement which can detect both of the external and internal quality of the product. Sugar content is an important internal quality attribute for any fresh fruit. This research work focuses on evaluating the use of hyperspectral imaging technique which employs the wavelength range of 685-900 nm for detecting the quality of apple based on sugar content. The partial least square (PLS) method has the potential to produce the calibration and prediction model from their spectra. It was found that the optimal spectral range for sugar content of apple was 704.48-805.26 nm and the PLS calibration model for sugar content determination needed 4 PLS factors under standard normal variate (SNV) preprocessing method. The correlation coefficient (R) between the hyperspectral imaging prediction results and reference measurement results was equal to 0.90749. The PLS algorithm produced the calibration models which gave reasonably good correlation for estimating the sugar content of apple. It can thus be concluded that hyperspectral imaging technique is potentially useful for assessing sugar content of apple.

Keywords: hyperspectral imaging technique, apple, sugar content, PLS

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Introduction

Non-destructive evaluation of the internal quality of fruits is an important field of research to improve their export potential. Determination of sugar content is a key factor to the export of fresh fruits. Sugar content is also one of the important parameters used for assessing apple quality. It may be determined from the juice extracted from the fruit flesh using refractometric method. However, this method of measurement is destructive, inefficient or time consuming, and prone to operational error. Unsuitable use of sugar content determination method for apple will result in less market value. The current method of apple sorting and grading for export market needs to be improved with more scientific approach. This issue can be addressed by evaluating the internal quality of the fruit non-destructively. Various researchers have reported on the development of different nondestructive sensing techniques for assessing postharvest quality of horticultural crops; they include mechanical force/deformation, sonic, impact, optical, and electrical techniques [1]. Most of these have not been adopted commercially because they do not correlate well with standard destructive measurements, nor meet the online sorting and grading requirements.

Exploring the possibility of using the optical property of fruits and vegetables for food product quality evaluation is gaining momentum. One example is near-infrared spectroscopy (NIRS), which has become a useful technique for measuring fruit internal quality, especially soluble solid content. Applications of NIRS have already been found for quality evaluation of food products, fruits and vegetables. Quality evaluation with the visible/near-infrared (VIS/NIR) region of spectra is being used to extract information from a small area or point from the food object. Considerable researches have reported on using NIRS to measure fruit internal quality such as sugar content or soluble solid content [2-8]. NIRS has also been demonstrated to have the potential for measuring other related flavour attributes for apple and other fruits [9-11]. Commercial NIRS systems are recently available for sorting and grading of fresh fruits. NIRS was also used for measuring fruit firmness [12-14] and other properties such as acidity [15-16]; however, the results are much less satisfactory. Also, NIRS is still unable to provide consistent and accurate measurement of other quality attributes such as fruit firmness [17-18]. Recently, hyperspectral or multispectral imaging technique was investigated for measuring fruit firmness and sugar content [19]. This new approach resulted in better prediction of fruit qualities than that by NIRS.

Hyperspectral imaging or imaging spectroscopy has its own advantage of extracting spectral information from a larger area of the object to provide more detailed information of the whole object. Application of this technique would be very useful for a more precise evaluation of internal properties of fruits. Lu and Peng [20] adopted the technique for predicting firmness of peach in the wavelength range of 500-1,000 nm. They found the best predictions at the wavelength of 677 nm for fruit firmness. Martinsen and Schaare [21] suggested 900-930 nm as important wavelength range for estimating soluble solid concentration of kiwi fruit. Dull et al. [22] selected 913 nm for predicting soluble solid content in sliced cantaloupe. Paliwal et al. [23] used different neural network models to classify cereal grains of five types based on their morphological character. The four-layer back-propagation network model classified the wheat and oat grains with satisfactory accuracy.

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Noh and Lu [24] evaluated firmness and soluble solid content of Golden Delicious apple by hyperspectral reflectance scattering and fluorescence. Principle component analysis and neural network model were used for the prediction of firmness and soluble solid. Better prediction results were obtained with the model which combined both the fluorescence and reflectance value with a correlation of 0.75 and standard error of prediction of 6.97 N.

The objectives of this study are to evaluate another internal quality attribute, viz. sugar content based on the spectral information collected using a hyperspectral imaging system, to select the best wavelengths for the determination of sugar content, and to develop a prediction model for predicting these sugar content parameters of apples from their spectral information.

Materials and methods

Sample preparation

The fruit samples for the study were purchased from a local supermarket in Zhenjiang City of China. One hundred fruits of "Fuji" apple cultivar were used for the experiment. They were transported to the laboratory and were stored at room temperature (20 ^{o}C) for 3 hours before being measured. The apples were divided into two groups. The first group (60 apples) was used as the calibration set for building the calibration model, and the second group (40 apples) was used as the prediction set for testing the robustness of predictive models. Hyperspectral image acquisition and analysis was conducted on 4 positions at the opposite sides of each apple equator. After the image acquisition, the fruit was subjected to destructive analysis by the refractometer to determine the sugar content. All these analyses were done on peeled apples.

Hyperspectral imaging system and data acquisition

The hyperspectral image data were acquired through a hyperspectral imaging system, Figure 1(a), which was developed by the Agricultural Product Processing and Storage Laboratory at Jiangsu University. Figure 1(b) shows a sketch of the system consisting of a hyperspectral imaging camera set (with complementary metal oxide semiconductor (CMOS) camera, prism-grating-prism assembly, and C-mount lens), a motion controller, a motorised positioning table, fibre-optic illuminators, a light source, and a computer.

The whole system was divided into three modules: the sensor module, the lighting source module and the conveyer module. The sensor module was a Specim Hyperspectral Imaging Camera (ImSpector V10E, Specim Spectral Imaging Ltd., Oulu, Finland). It is an original equipment manufacturer (OEM) product which included a back-illuminated CMOS camera, a spectrograph with a prism-grating-prism construction and a C-mount lens which was attached to the CMOS camera. The spectral range was 408–1,117 nm with a nominal spectral resolution of 2.8 nm. For the lighting source module, two 150-W quartz-halogen DC stabilised fibre-optic illuminators (Fiber-Lite DC950 Illuminator, Dolan-Jenner Industries Inc, MA, USA) were used. The conveyer module consisted of an auto-translation stage or motorised positioning table (TSA200-A, Zolix Instruments Co., Ltd., Beijing, P.R. China) and a motion controller (SC300, Zolix Instruments Co., Ltd.). Thus, the motorised drive moves the lens assembly via the translation floor so that successive lines of the target are scanned while the target itself remains stationary.

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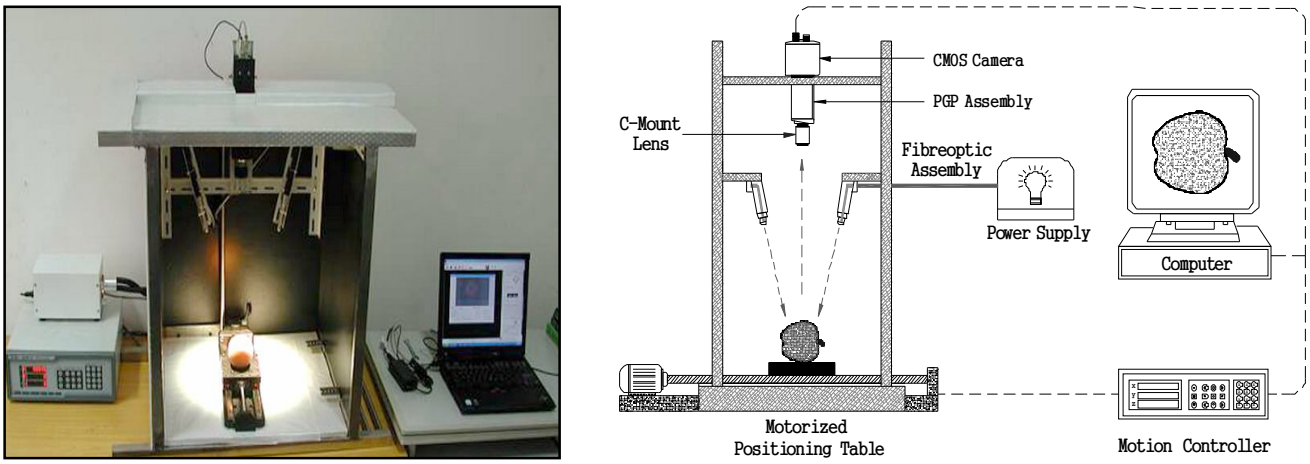

(a) (b)

Figure 1. (a) Hyperspectral imaging system developed at the Agricultural Product Processing and Storage Laboratory, Jiangsu University, in Zhenjiang, P.R. China, (b) Sketch of the system

The apple sample was put on the platform of the translation floor and the acquisition of the hyperspectral imaging data was started. The hyperspectral imaging was captured line by line as the translation floor incrementally moved the samples through the field of view of the hyperspectral imaging camera under reflected light at wavelengths ranging from 408 nm to 1,117 nm with 0.67 nm intervals, which resulted in 1,024 spectral wavebands. The image data acquired by the hyperspectral imaging system were arranged with a spatial dimension of 1,280×600 pixels and with 1,024 spectral bands from 408 to 1,117 nm, as shown in Figure 2.

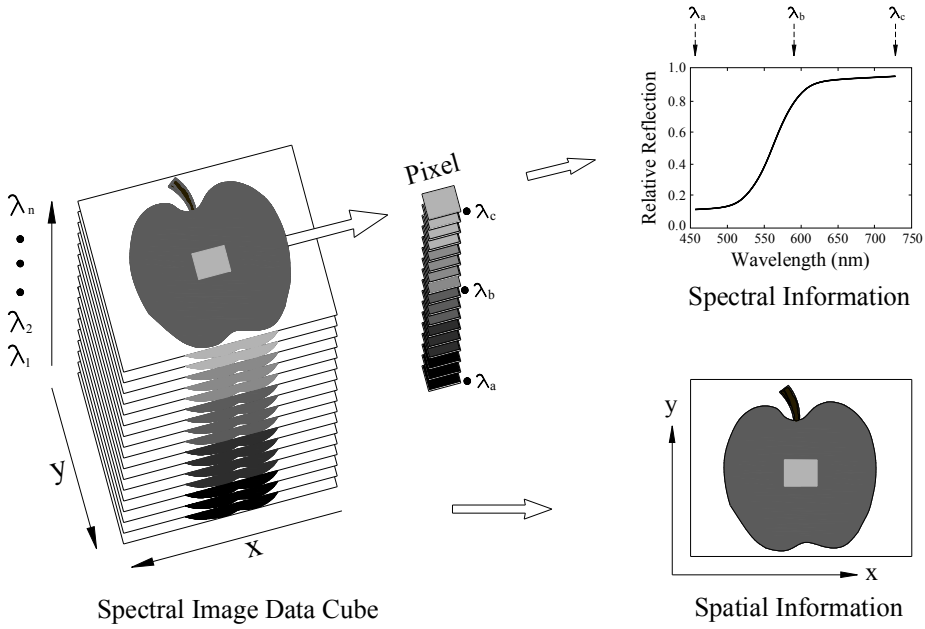

Figure 2. A three-dimension imaging cube (x, y, λ) acquired by the hyperspectral imaging system with two spatial dimensions (x, y) and one spectral dimension (λ)

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Hyperspectral imaging data preprocessing

The hyperspectral images were firstly corrected with a white and a dark reference. The dark reference was used to remove the effect of the dark current of the CMOS detector, which is thermally sensitive [25-29]. The corrected image (R) was estimated using Eq. 1:

$$R = \frac{I - B}{W - B} \qquad (1)$$

where I is the recorded hyperspectral image, B is the dark image (approximately 0% reflectance) recorded by turning off the lighting source with the lens of the camera completely closed, and W is the white reference imaging obtained by a white Spectralon panel (approximately 99% reflectance). Spectral reflectance R values range from 0 to 1.

The original spectral profile data of 100 apple samples are shown in Figure 3. In this research, spectral profiles of apple samples from 685-900 nm were used for analysis. Partial least square (PLS) algorithm of multivariate calibration was attempted several times to determine the optimal spectral range for constructing the calibration model. It was found that the optimal spectral range for sugar content of apples was 704.48-805.26 nm.

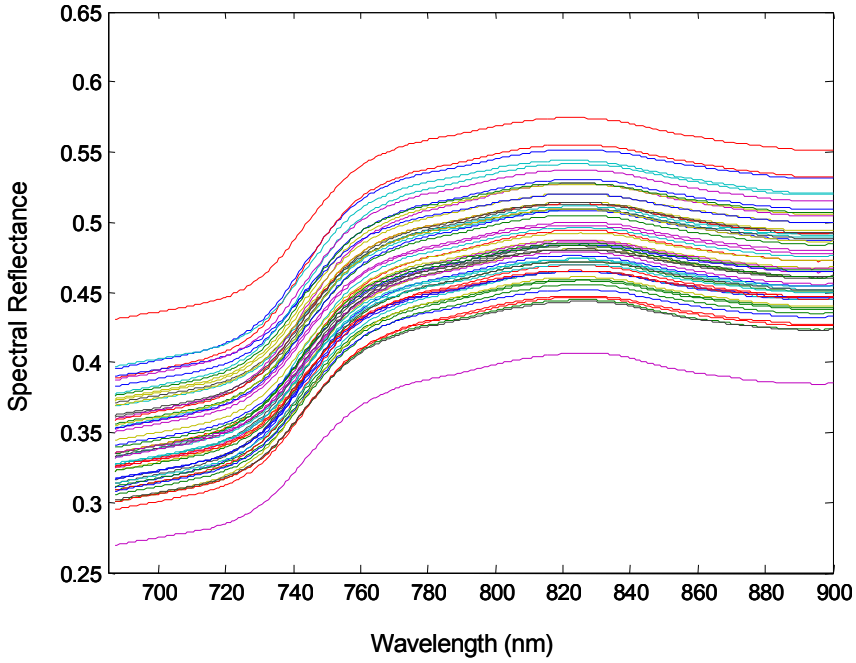

Figure 3. Original spectral profiles of apple samples from 685 nm to 900 nm

Sugar content reference measurement

A sugar refractometer (Model 2WA-J Abbe Refractometer, Shanghai Optical Instrument, China) reading in [°Brix] units with an accuracy of ±0.2% and a sugar content scale between 0-95% Brix was used for the determination of sugar content. A small sample of apple juice was used to measure the refractive index by the refractometer. The sugar content determination was performed on the same 4 positions as those used for hyperspectral imaging measurement on each apple sample around the equator. The mean value of sugar content was the average of 4 measurements.

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Spectral preprocessing analysis

The spectral data were analysed using PLS regression with preprocessing. Four spectral preprocessing methods were applied comparatively: these were standard normal variate (SNV), mean centring, multiplicative scatter correction (MSC) and min/max normalisation.

First, SNV, a mathematical transformation method of the spectra, was used to remove slope variation and to correct for scatter effects. Each spectrum was corrected individually by first centring the spectral values, then, the centred spectrum was scaled by the standard deviation calculated from the individual spectral values. Second, the mean centring method process was to calculate the average spectrum of the data set and subtract that average from each spectrum. Third, MSC was used for the correction of scattered light on the basis of different particle sizes. The technique was used for correcting the additive and multiplicative effects in the spectra. Finally, min/max normalisation, a type of normalisation, was utilised to transform the data into a preferred range, in which the minimum value of an attribute was substracted from each value of the attribute and then the difference divided by the range of the attribute [30-31]. In general, the range of the spectral value after min/max normalisation spectral preprocessing is set [0 1]. Figure 4 shows the results of the different methods after preprocessing.

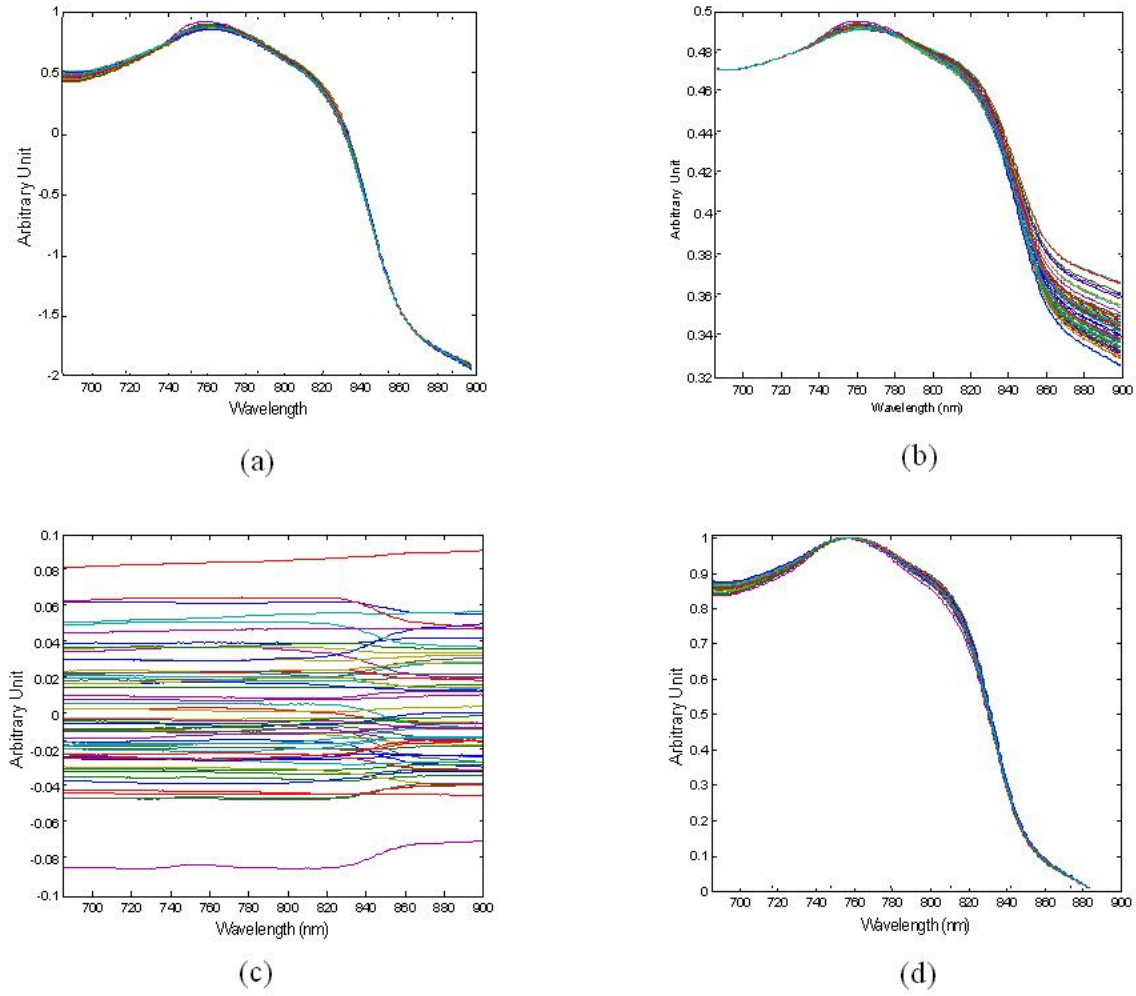

Figure 4. Preprocessing reflectance spectra of apples: (a) SNV, (b) MSC, (c) mean centring, and (d) min/max normalisation

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Software

For the hyperspectral imaging data acquisition, Spectral Cube（Spectral Imaging Ltd., Finland, AutoVision Inc., CA, USA） was used. All data processing and analysis were performed with environment for visualising images (ENVI) V.4.3 (Research Systems Inc., Boulder Co., USA) and Matlab Version.7.0 (Mathworks, Natick, USA) for Windows XP.

Results and Discussion

Quantitative analysis of the PLS model

One hundred "Fuji" apples were selected to construct the PLS model in this experiment. All 100 spectra were divided into 2 sets, i.e. the calibration set and the prediction set. To avoid bias in the subset selection, the division was arranged as follows: all samples were sorted according to their respective y-value, viz. the reference measurement value of sugar content. In order to come to a 3/2 division of calibration/prediction spectra, three spectra from every five samples were selected for the calibration set, and two for the prediction set so that finally the calibration set contained 60 spectra (Table 1). The remaining 40 spectra constituted the prediction set (Table 2). As seen in Tables 1 and 2, the range of y values in the calibration set covers that in the test set, therefore the distribution of the samples was appropriate in the calibration and prediction sets.

Table 1. Reference measurements and sample numbers in calibration set

Component	Unit	S.N.	Range	Mean	S.D.
Sugar content	^{o}Brix	60	7.20-17.45	12.50	1.75

S.N. = sample number; S.D. = standard deviation, ^{o}Brix = sugar content unit

Table 2. Reference measurements and sample numbers in prediction set

Component	Unit	S.N.	Range	Mean	S.D.
Sugar content	^{o}Brix	40	8.52-15.60	12.51	1.61

S.N. = sample number; S.D. = standard deviation, ^{o}Brix = sugar content unit

The performance of the final PLS model was evaluated in terms of the root mean square error of cross-validation (RMSECV), the root mean square error of prediction (RMSEP), and the correlation coefficient (R). For RMSECV, a leave-one-sample-out cross-validation was performed: the spectrum of one sample of the calibration set was deleted from this set and a PLS model was built with the remaining spectra of the prediction set [32]. The left-out sample was predicted with this model and the procedure was repeated by leaving out each of the samples of the calibration set. RMSECV was calculated according to Eq.2:

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{n}\left(\hat{y}_{\setminus i} - y_i\right)^2}{n}} \qquad (2)$$

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where n is the number of samples in the calibration set, y_i is the reference measurement result for sample i, and $\hat{y}_i$ is the estimated result for sample i when the model is constructed with sample i removed. The number of PLS factors included in the model was chosen according to the lowest RMSECV. This procedure was repeated for each of the preprocessed spectra. For the prediction set, RMSEP was calculated according to Eq. 3:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}\left(y_i - \hat{y}_i\right)^2}{n}} \qquad (3)$$

where n is the number of apple samples in the prediction set, y_i is the reference measurement result for prediction set sample i, and $\hat{y}_i$ is the estimated result of the model for prediction sample i. Finally, the model with the overall lowest RMSECV was selected as the final model. Correlation coefficients between the predicted and the measured value were calculated for both the training and the test set according to Eq. 4:

$$R = \sqrt{1 - \frac{\sum_{i=1}^{n}\left(\hat{y}_i - y_i\right)^2}{\sum_{i=1}^{n}\left(y_i - \bar{y}_i\right)^2}} \qquad (4)$$

Sugar content

In the application of PLS algorithm, it is generally known that the spectral preprocessing methods and the number of PLS factors are deciding parameters. The optimum number of factors is determined by the lowest RMSECV. Figure 5 shows the RMSECV plotted as a function of PLS factors for determining the sugar content with different spectral preprocessing methods, i.e. SNV, MSC, mean centring and min/max normalisation. It was found that the RMSECV of SNV and min/max method decreased sharply together in the same trend, while the RMSECV of MSC and mean centring method were higher than the former.

However, in comparing between the four spectral preprocessing methods, it was found that the SNV spectral preprocessing method exhibited the best result, and when the number of principal components (PCs) was equal to 4, the value of RMSECV was lowest. Therefore, the best predictive model was achieved with 4 PLS factors after the SNV spectral preprocessing method.

Table 3 shows the best results of the calibration models by different spectral preprocessing methods for determining sugar content. Apparently, the lowest RMSECV, equal to 0.70699 ^{o}Brix, was obtained with the SNV spectral preprocessing method, which needed 4 PLS factors. In this application then, the SNV seemed to perform better than other preprocessing methods.

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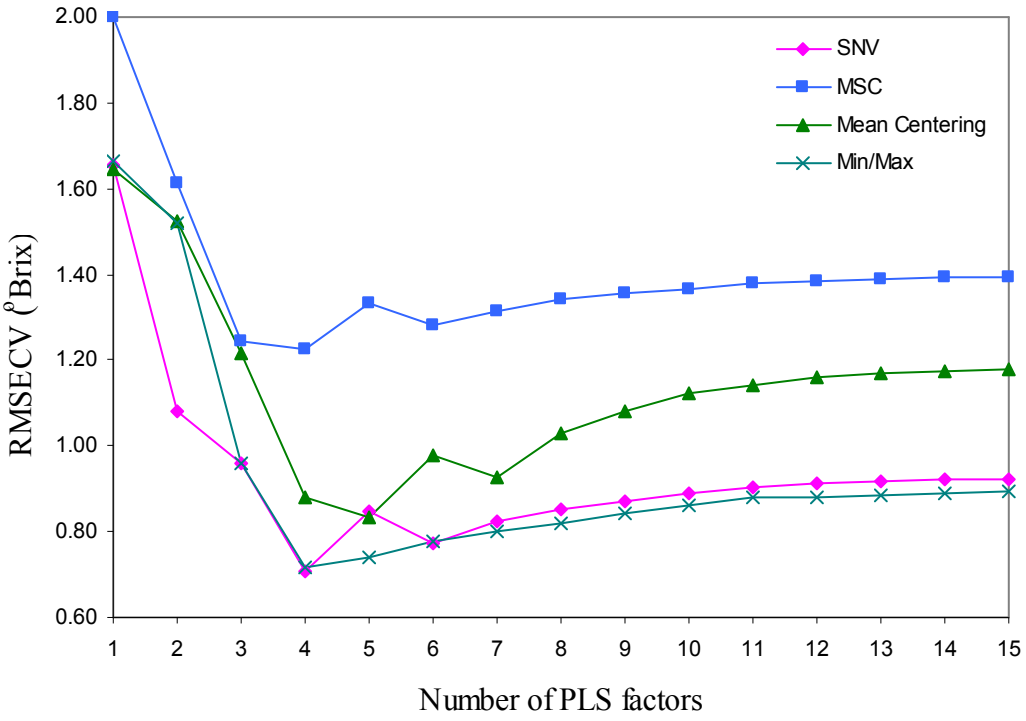

Figure 5. Effect of number of PLS factors on RMSECV for sugar content calibration model

Table 3. Best results for each of the processing method for the models of sugar content

Preprocessing method	PLS factors	RMSECV (°Brix)	RMSEP (°Brix)	R (calibration)	R (prediction)
SNV	4	0.70699	0.66942	0.89771	0.90749
MSC	4	1.22370	0.90198	0.64691	0.82438
Mean centring	5	0.83328	0.85155	0.85461	0.84525
Min/max normalisation	4	0.71608	0.67512	0.88946	0.90029

Figure 6 gives a scatter plot showing a correlation between hyperspectral imaging prediction value and reference measurement of sugar content by the SNV spectral preprocessing method. All blue dots represent prediction data, which are in good correlation with the reference measurement data; many points fall on or close to the unity line. Sugar content in the prediction set was predicted with the RMSEP value of 0.66942 °Brix. The correlation coefficients for this calibration model are equal to 0.89771 and 0.90749 for the calibration and prediction set respectively.

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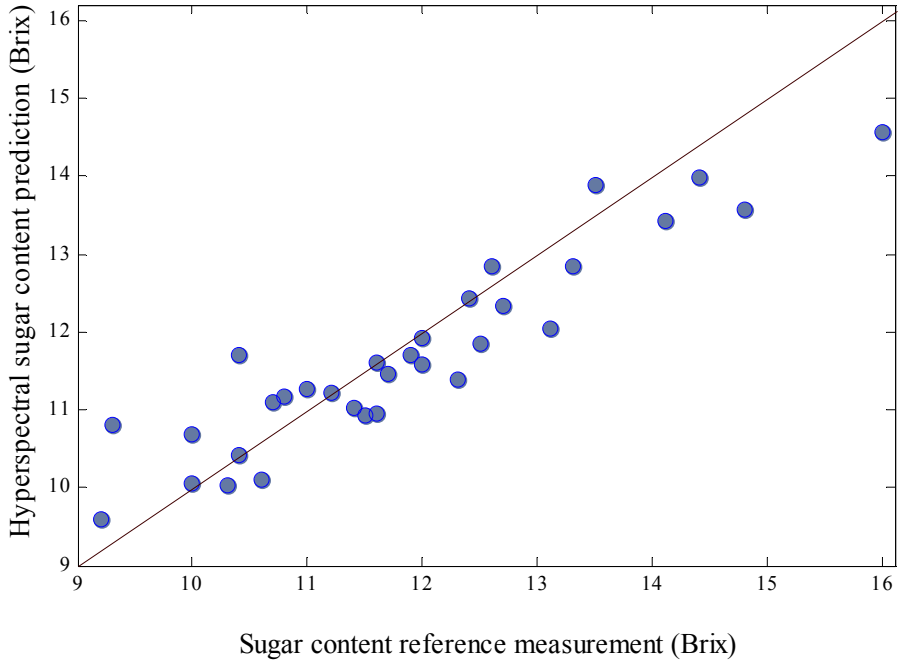

Figure 6. Reference determinations versus hyperspectral prediction for sugar content of calibration set data

Conclusions

The study has shown the potential application of hyperspectral imaging technique for the prediction of sugar content of apple fruits. The technique employed the wavelength range of 685-900 nm for estimating the sugar content. It was found that the optimal spectral range for sugar content of apples was 704.48-805.26 nm. The PLS method has the potential to estimate the calibration and prediction model from their spectra. The PLS calibration model for sugar content determination was found to need 4 PLS factors under the SNV preprocessing method. The correlation coefficient (R) between the hyperspectral imaging prediction results and the reference measurement results was equal to 0.90749. The PLS algorithm produced the calibration models which gave a reasonably good correlation for estimating the sugar content of apple.

Acknowledgements

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Report

Vegetation and vascular flora of the Mekong River, Kratie and Steung Treng Provinces, Cambodia

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Abstract: A preliminary and detailed botanical survey of the islands in the Mekong River between Kratie and Steung Treng was done. This area includes the most biologically intact and threatened riparian and terrestrial ecosystems along the river in Cambodia. The vegetation includes six riverine zones and four terrestrial facies. Riverine habitats are mostly intact while the terrestrial vegetation ranges from destroyed to degraded. Effective conservation measures are required to stop further habitat destruction and loss of biodiversity. One new species, 23 records for the Cambodian flora, and a total of 690 species were collected. Detailed descriptions of all habitats, a database, and photographs are included. Increased exploitative human settlement in the area has caused drastic environmental changes with extensive deforestation and hunting. The forests are grazed, burned, logged, and often cleared for agricultural use without effective control. Sustainable management and scientifically acceptable development must be implemented before the area is totally ruined. Properly conceived reforestation is urgently required as well as a conservation education project aimed directly at the people living in the area. Unless effective restraints are implemented the area will become biologically destitute and will not be able to provide the natural resources that people require--in short, the area will become uninhabitable. Restoration of degraded or destroyed places will be impossible or far more difficult than conservation and intelligent management of presently endangered places. The potential for profitable eco-tourism should also be considered since tourists will certainly want to visit natural ecosystems on some of the islands. Only if local people are directly involved in eco-tourism and understand the necessity of conservation can this activity be successful. It is strongly recommended that continued botanical research be conducted in the area in order to more fully understand the distribution and abundance of the plants there.

Keywords: botanical survey, vascular flora, Mekong River, Cambodia

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Introduction

Background

Gagnepain [1] provides detailed information concerning the itineraries and biographies of pioneer French plant collecting in Indochina. Four people are known to have collected along the Mekong River between Kratie, Cambodia and Khone Island, Lao PDR. Their specimens are in the Paris Herbarium.

Clovis Thorel (1833-1911) [1] a physician-botanist, collected the first plant specimens along the Mekong River in Cambodia and Laos during 1866-1868. J.B.L. Pierre (1833-1905) [1], director of the Botanic Gardens, Saigon (1865-1877), collected extensively in Cambodia and especially along the Mekong River from Phnom Penh to Khone Island, Lao PDR. Pierre produced the 5-volume Flore Forestière de Cochinchine (1879-1907). François Harmand (1845-1921) [1] collected in Indochina during 1875-1877, including along the Mekong River at Kratie. Eugene Poilane (1887-1964) [1], from the Paris Herbarium, made collections in Indochina during 1917-1936 and along the Mekong River from Kratie to Khone Island.

Maxwell [2,3] compiled a flora for the Seephandon area, southern Lao PDR during 1997-1998. His survey resulted in 131 families and 731 species of vascular plants along with a detailed plant database and vegetation map [3]. An unpublished and quite incomplete report by Meng Monyrak for IUCN listed 102 vascular plants in a Ramsar conservation site above Steung Treng. The material was identified by Maxwell and is deposited in CMU Herbarium. R. Timmins (2007), a zoologist, surveyed this Ramsar site and included a chapter on vegetation and wildlife habitats. His comprehension of the vegetation there was rudimentary while his terminology was totally unacceptable and should be ignored.

Our plant team found 120 families and 683 species of vascular plants as well as 7 bryophytes during this survey. An extensive plant database, a vegetation map, profiles of the vegetation, and photographs of the various habitats and plants are included.

Location

A section *c.* 55 km long of the Mekong River from Sambour in the northern part of Kratie Province to the southern part of Siem Bok District, Steung Treng Province was studied (13° 17’ 55’’- 13° 4’ 47’’ N latitude and 105° 56’ 49’’- 106° 13’ 47’’ E longitude, see Map 1). This part of the river is braided and has over 40 ‘’permanent’’ islands, 18 being over 3 km long, with Rongnieu Island being the largest at 37 km long and 5 km wide. The islands are long, narrow, and have channels between them, the most extensive being 11 km wide. Other islands are seasonally submerged and can only be visited during the cool/dry and hot/dry seasons when the river level is low.

Within the study area there are 9 permanent villages with an estimated population of 5,553 in 2005 [2]. The overall population density of the area is low (0-70 people per km²), but improved access has encouraged more outsiders to exploit the area, especially when the river level is low.

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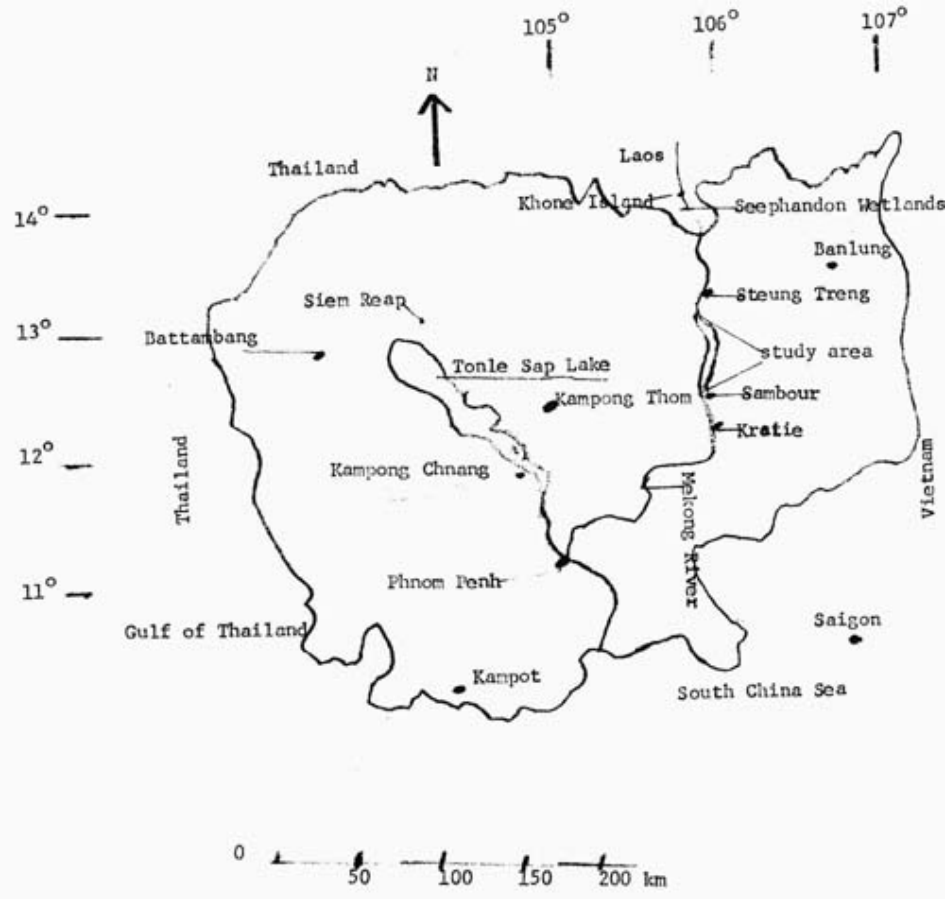

Map 1. Map of Cambodia showing the Mekong River and the study area between Kratie and Steung Treng

Geology

The bedrock is mostly metamorphic sandstone (paragneiss) which was originally from sand deposits in the sea and compressed to form sandstone and more recently metamorphosed. The age of the rocks is Upper Triassic-Lower Jurassic, *c.* 180-200 million years [2]. Shale of similar age is also found in some areas. Riverine deposits of silt, clay, sand, and gravel are recent Quaternary deposits. Thin layers of fresh water limestone (tufa), of recent origin, were also infrequently seen. The elevation of land exposed during the lowest levels of the river was *c.* 20 m, while the islands, all flat, were *c.* 30 m elevation.

Climate

The climate of Cambodia is distinctly seasonal with three basic seasons, viz. dry/cool, dry/hot, and rainy. The NE monsoon results in dryness from November to April. The first part of this period is cool, while the latter two months are the hottest time of the year. The SW monsoon causes the rainy season from May to October.

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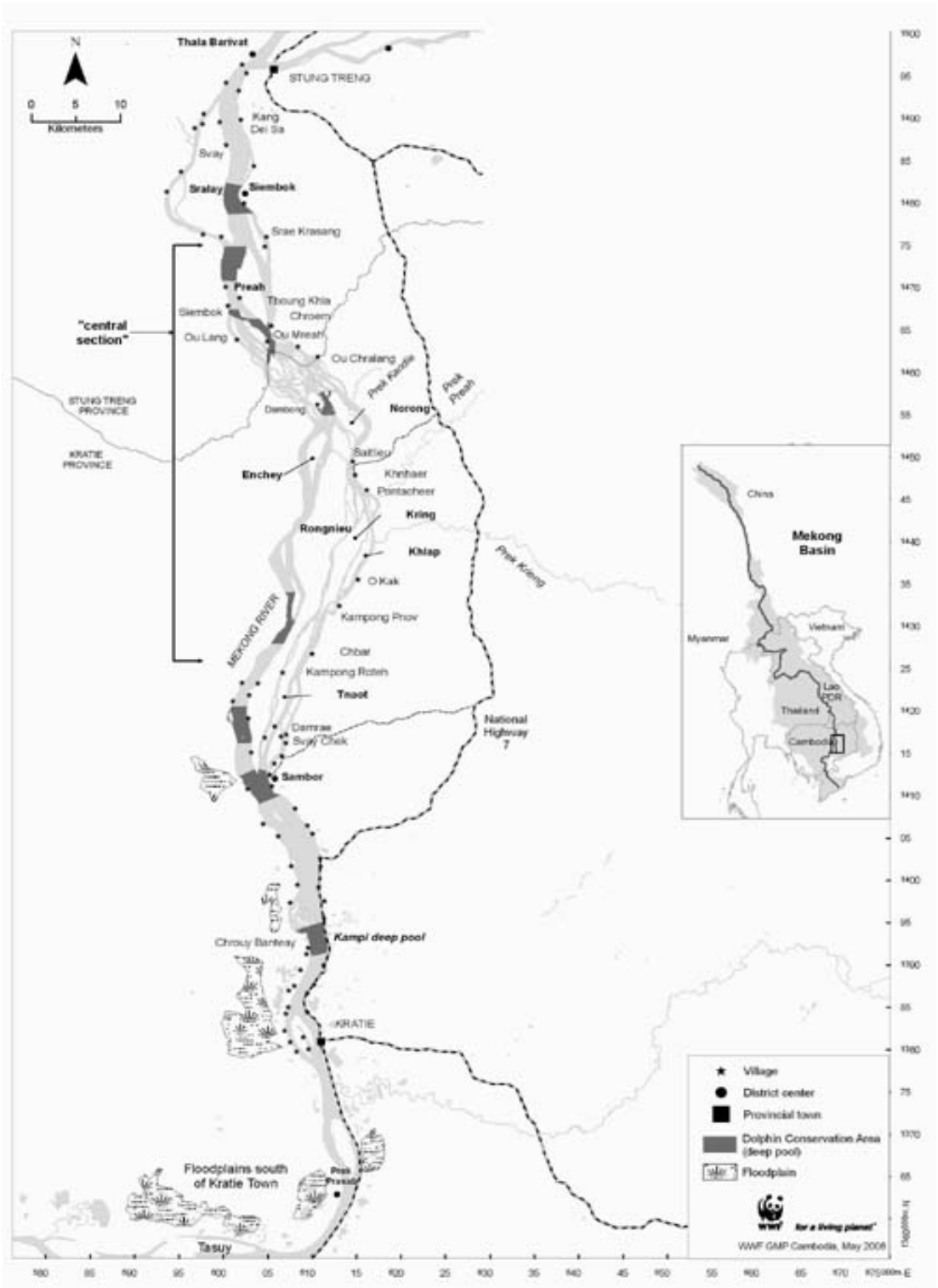

Map 2. Details of the study area shoeing some of the islands surveyed (central section). Source: WWF Cambodia

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The mean annual temperature at Steung Treng during 2003-2004 was 23.5-34° C (lowest 11.5° C, highest 40° C). Annual rainfall at Steung Treng from 1994-2000 ranged from 1441-2600 (average 1966) mm. During 1997-2000 there was as average of 2050 mm of rainfall at Kratie, ranging from 1743-2549 mm per year. In general the lowest amount of rain is in January (0.0-0.9 mm) and the most in September (333-469 mm).

The river level directly corresponds to the amount of rainfall and has an average monthly discharge of 2200 m^3/second (April) to 36,700 m^3/second (September) at Kratie.

Research trips and fieldwork

Three trips were made to the study area which corresponded to the three different seasons affecting the area, viz.

1. 10-23 November 2006, dry/cool season, river level receding;
2. 10-25 March 2007, dry/hot season, river level nearly the lowest; and
3. 29 July-13 August 2007, rainy season, river level almost maximum.

This research was organised by World Wildlife Fund (WWF) Cambodia and included four research teams, viz. plant, fish, amphibians + reptiles, and birds + mammals. The overall result of this work has been compiled by Bezuijen et al. [2].

Considering the size of the study area an opportunistic approach of collecting specimens was pursued in which as many islands were visited as possible, including the mainland. Basically every flowering and fruiting species was collected, while non-reproducing plants were identified in the field and recorded, and notes on vegetation types were made.

Well over 700 specimens were collected and identified in Chiang Mai University Herbarium (CMU). An enumeration of all recorded vascular plants and bryophytes is shown in Appendix 1. The following section gives a detailed description of the vegetation types present in the study area.

Vegetation Types

There are two main kinds of vegetation in the study area, *viz*. riverine (riparian) and terrestrial, i.e. on land above the flood level of the river. The riverine vegetation includes all vegetation in the river to the highest water level attained in August-September. This area is controlled by the Fisheries Department, while terrestrial areas are regulated by the Forestry Department (Figures 1 and 2).

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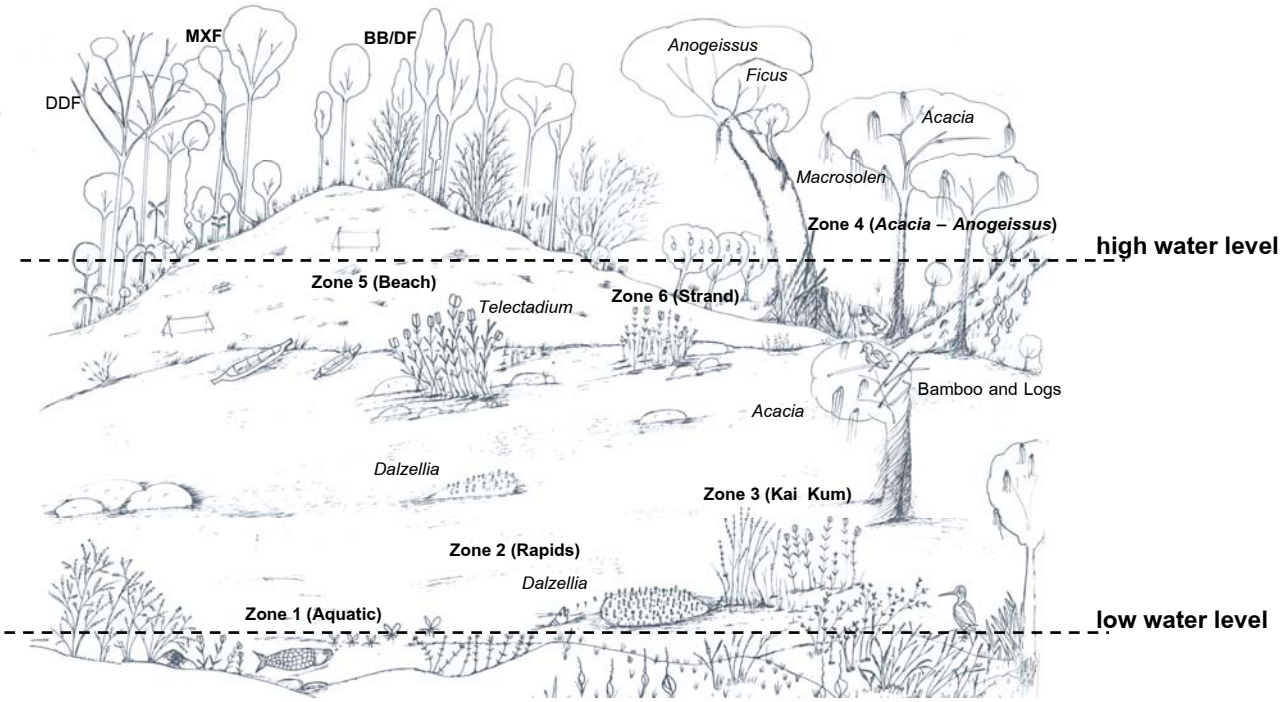

Figure 1. Riparian vegetation zones and forests in the study area (Drawing by P. Palee).

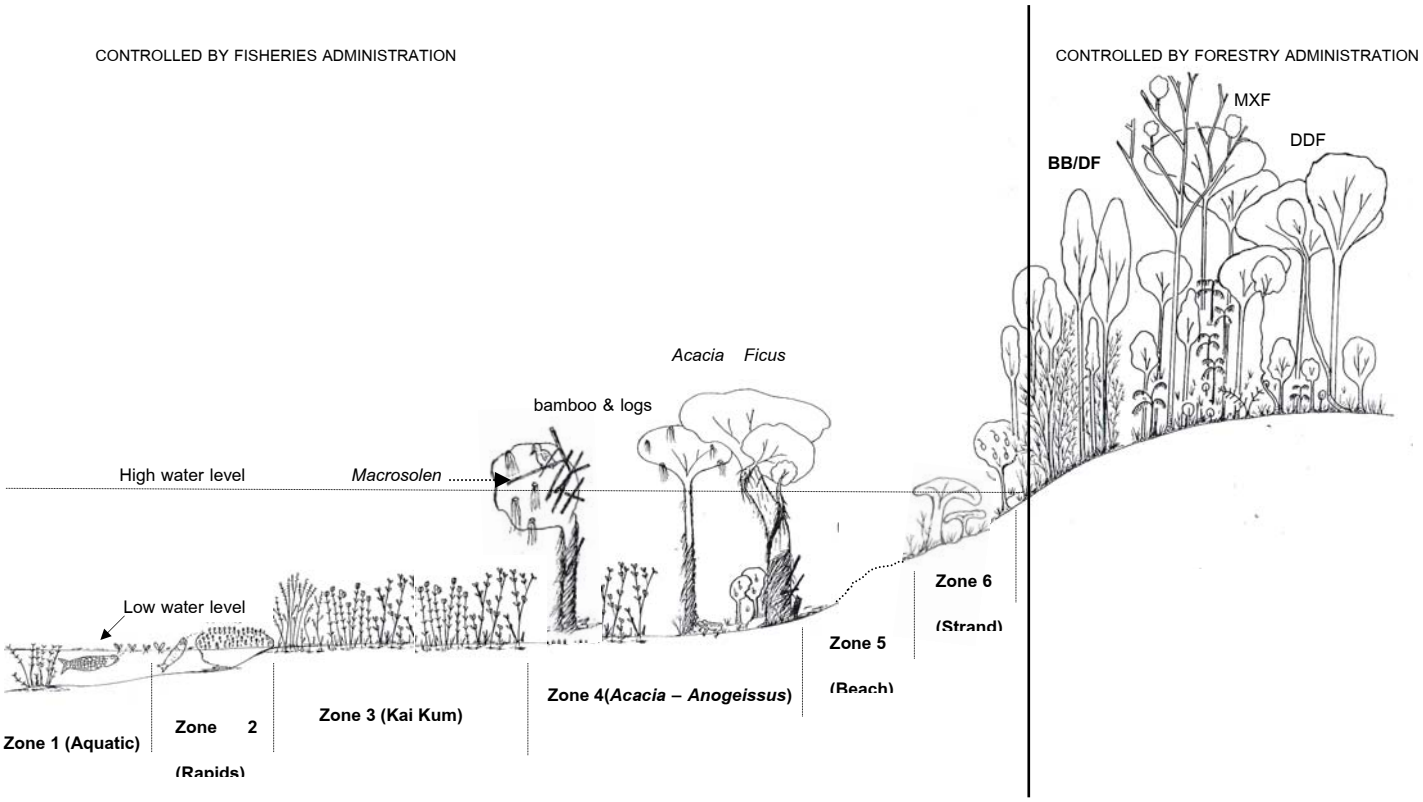

Figure 2. Riparian vegetation zones and forests in the study area Drawing by P. Palee

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Riverine (Riparian) Vegetation

The Mekong River, due to its immense size, great fluctuations of water (up to 10 m in the study area), and particular geomorphology, has developed a distinct and very diverse riverine vegetation in the area between Kratie and Stung Treng. Six vegetation zones have been distinguished in this system. All of these zones are exposed during the lowest level of the river during February-May and only the uppermost zone can be seen, in part, during August-September when the water level is the highest. These six zones are not always apparent in many areas due to the absence of bedrock which is vital for the development and stability of some zones. Shifting sandbars and ephemeral beach formations also tend to cause variation in the extent of some zones. Erosion of the margins of some islands has resulted in a steep drawdown area in which the upper riverine zones are often not present. Bedrock, essential for Zones 2-4, is often absent, thus these places usually have sand extending to the terrestrial vegetation (Photo 1, Appendix 1).

The five zones above the aquatic (river) zone include species which are both amphibious and seasonally rheophytic. The vegetation ranges from delicate annual aquatic herbs to trees up to 15 m tall. Many species found in the riverine vegetation are only known from the Mekong River. The vegetation tends to increase in height, density, and diversity from the lowest level of the river (*c.* 20 m elevation) to the terrestrial vegetation (*c.* 30 m).

Zone 1: Aquatic

The aquatic plants here are all herbs and are readily found in the river during the dry season when the water level is lowest, the flow slowest, and quality clearest. These plants are either floating or submerged and attached to the bottom, often on rocks. All are obligate aquatics and cannot survive without water. *Potamogeton crispus* L. (Potamogetonaceae), *Najas indica* (Willd.) Cham. (Najadaceae), *Hydrilla verticillata* (L.) Roy., and *Vallisneria gigantea* Greab. (both Hydrocharitaceae), all monocots, are prevalent. *Ceratophyllum demersum* L. (Ceratophyllaceae) was the only dicot found. Algae were not collected during this project.

Zone 2: Rapids ("Boong")

I referred to this zone in the Seephandon wetlands, Laos, which is *c.* 90 km north along the Mekong River, as "Boong", which is the Lao name for this open, rocky, sparsely vegetated habitat [3,4]. This is the rocky to sandy area immediately above the aquatic zone with vegetation that is the first to be submerged and last to be exposed in the annual cycle of the river (Photo 2, 3). It consists of several deciduous herbs and shrubs, often scattered, with a general lack of trees. Herbs are common with *Fimbristylis cymosa* R. Br. (Cyperaceae), *Cryptocoryne crispatula* Engl. var. *crispatula* (Araceae), which is a new record for the Cambodian flora; and the edible Pteridophyte *Diplazium esculentum* (Retz.) Sw. (Athyriaceae). Shrubs, all deciduous, amphibious rheophytes, are mostly epilithic and grow in dense clusters in rocky places. *Telectadium edule* H. Baill. (Asclepiadaceae)**,**

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Homonoia riparia Lour. and *Phyllanthus jullienii* Beille (both Euphorbiaceae), and *Xantonnea parviflora* (O. K.) Craib var. *salicifolia* (Pierre *ex* Pit.) Craib (Rubiaceae) are common shrubs. *Crateva magna* (Lour.) DC. (Capparaceae), a shrub or treelet, is also found here, but of lesser stature and frequency as in Zones 4 and 5. *Dalzellia carinata* (Lec.) C. Cuss. (Tristichaceae) is a tiny, epilithic, moss-like herb which grows in dense clusters on rocks in areas with a fast current close to the water level. This species was found in flower in March and is remarkable due its ability to survive in such an extreme habitat.

Zone 3: "Kai Kum"

"Kai Kum" is the Lao name for *Phyllanthus jullienii*, which dominates this zone in the Seephandon wetlands [3,4]. Places above Zone 2, which generally have more plant diversity and abundance, as well as more vigorous growth, are included here. Water flow is less rapid here and in some instances Zone 2 merges with Zone 3—a clear distinction being difficult to make. This zone has several shrubs which are usually found in Zone 2, e.g. *Morinda pandurifolia* O.K. var. *oblonga* (Pit.) Craib (Rubiaceae), *Blachia siamensis* Gagnep. (Euphorbiaceae), and *Paravitex* sp. (Verbenaceae). *Homonoia riparia* is common, but *Telectadium edule* is mostly absent. *Oxystelma esculentum* (L. f.) R. Br. (Asclepiadaceae), a vine, as well as most of the herbs found in Zone 2 are also present. The first trees are found here and include *Barringtonia acutangula* (L.) Gaertn. (Lecythidaceae), *Eugenia mekongensis* Gagnep. (Myrtaceae), and an occasional *Crateva magna.*

Zone 4: Acacia- Anogeissus

This zone is characterised by two seasonally rheophytic, deciduous trees which only grow in rocky places above Zone 3, viz. *Acacia harmandiana* (Pierre) Gagnep. (Leguminosae, Mimosoideae), and *Anogeissus rivularis* (Gagnep.) Lec. (Combretaceae)—both of which can grow up to 15 m tall and become partly to completely submerged during August-September. Their crowns are frequently bent downstream by the strong river current and collect various debris (logs, bamboo, trash, etc.) which remain in place during the dry season (Photo 4). Both species develop thick mats of fibrous, black adventitious roots in the lower 2-4 m of the trunk which are also bent downstream. These two species are hosts for *Macrosolen cochinchinensis* (Lour.) Tiegh. (Loranthaceae), a common epiphytic, hemi-parasitic shrub on the upper branches. Several species of *Ficus* (Moraceae), e.g. *F. benjamina* L., *F. rumphii* Bl., and *F. virens* Ait. (Photo 5) also grow as epiphytic trees on both of the dominating trees. Figs (synconia) produced by these and other species of *Ficus* are an important food source for many birds, various mammals, and fish.

This zone, often isolated or directly merging with terrestrial vegetation, also has some woody climbers that are absent from the lower zones. Some of these include: *Dalbergia volubilis* Roxb., *Paraderris elliptica* (Wall.) Adema, *Derris scandens* (Roxb.) Bth. (all Leguminosae, Papilionoideae), and *Hiptage triacantha* Pierre (Malpighiaceae). Herbs are common in this zone and often include some of those found in Zones 2 and 3. *Dichanthium caricosum* (L.) A. Camus (Gramineae); *Fimbristylis*

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brunneoides Kern and *F. jucunda* (Cl.) Kern (Cyperaceae)—monocots; *Hemigraphis modesta* R. Ben. (Acanthaceae), *Rotula aquatica* Lour. (Boraginaceae), and *Paravitex* sp. (Verbenaceae), both shrubs, and *Microcos sinuata* (Wall. *ex* Mast.) Burr. (Tiliaceae), a treelet, are also found here.

Mimosa pigra L. (Leguminosae, Mimosoideae), an invasive, naturalised, spiny, vigorous, herb-shrub from tropical America is rapidly becoming established from Zone 4 to the terrestrial areas. It is a noxious weed that develops dense growth at the expense of native vegetation and tolerates flooding, fire, and hacking. This species will become a very serious environmental problem in the future unless an effective eradication programme is established [5].

Zone 5: Beach

All open, sandy, seasonally inundated areas have been included in this zone. Sandbars, often isolated in the riverine area, as well as sandy areas above Zone 4, are common throughout the study area. Due to the lack of bedrock and sufficient organic nutrients, these sandy areas lack perennial, especially woody vegetation as found in Zones 2-4. Annual herbs, which germinate and produce seeds during October-July, are numerous, but usually very sparse in abundance. Many of these plants also colonise disturbed and agricultural areas and are considered as weeds. None are unique to this zone, but most of them do not inhabit the other riverine zones.

Both dicots and monocots are well-represented, but Pteridophytes (ferns) are absent. Some common dicots include: *Cleome viscosa* L. (Capparaceae), *Dentella repens* (L.) J. R. & G. Forst., and *Hedyotis pinifolia* Wall. *ex* G. Don (both Rubiaceae); *Eclipta prostrata* (L.) L. and *Grangea maderaspatana* (L.) Poir. (both Compositae); *Lindernia antipoda* (L.) Alst., *L. crustacea* (L.) F. Muell. var. *crustacea*, and *Scoparia dulcis* L. (all Scrophulariaceae); *Polygonum plebium* R. Br. (Polygonaceae), and *Phyla nodiflora* (L.) Greene (Verbenaceae). Monocots, especially Cyperaceae (sedges) and Gramineae (grasses) are also common. *Cyperus cuspidatus* Kunth, *Fimbristylis aestivalis* (Retz.) Vahl var. *aestivalis*, *F. dipascea* (Rottb.) Cl., and *F. jucunda* (Cl.) Kern (Cyperaceae) are frequently found. Some common Gramineae include: *Digitaria bicornis* (Lmk.) Roem. & Schult., *D. radicosa* (Presl) Miq., *Dactyloctenium aegyptium* (L.) P. Beauv., *Echinochloa colona* (L.) Link, *Leptochloa chinensis* (L.) Nees, and *Hemisorghum mekongense* (A. Camus) C.E. Hubb *ex* Bor—the latter being restricted to this zone and is a new record for the Cambodian flora.

Saccharum arundinaceum Retz. and to a lesser extent *S. spontaneum* L., both robust evergreen Gramineae, often form dense colonies on beaches close to the margins of terrestrial vegetation. These areas provide essential habitats for many animals and also help reduce erosion.

Zone 6: Strand

This is the highest riverine zone which is the last to be flooded and first to be exposed. It consists mainly of woody dicots and directly abuts terrestrial vegetation, sometimes without a distinct beach below it. In most instances, the vegetation here is dense, evergreen, and quite diverse. *Ficus heterophylla* L. f. (Moraceae) is a common creeping vine/woody climber found in this zone.

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Polyalthia modesta (Pierre) Fin. & Gagnep. (Annonaceae), a shrub, *Fluggea virosa* (Roxb. *ex* Willd.) Voigt (Euphorbiaceae), a treelet, and *Crateva magna*, a small tree, are common. Woody climbers include: *Ventilago harmandiana* Pierre (Rhamnaceae), *Derris scandens, Bauhinia bracteata* (Grah. *ex* Bth.) Baker ssp. *bracteata* (Leguminosae, Caesalpinioideae), *Combretum trifoliatum* Vent. (Combretaceae), and *Glossocarya siamensis* Craib (Verbenaceae). Trees are plentiful and form a closed, single canopy in most places. Many of these trees are restricted to this zone. Some common examples are: *Homalium brevidens* Gagnep. and *H. caryophyllaceum* (Zoll. & Mor.) Bth. (Flacourtiaceae), *Pterospermum diversifolium* Bl. (Sterculiaceae, Photo 6), *Quassia harmandiana* (Pierre) Noot. (Simaroubaceae), *Crudia chrysantha* (Pierre) K. Sch. (Leguminosae, Caesalpinioideae), *Combretum quadrangulare* Kurz (Combretaceae), *Cordia dichotoma* Forst. f. (Boraginaceae), *Mallotus* (*Trewia*) *nudiflorus* (L.) Kul. & Welz. (Euphorbiaceae), *Nauclea orientalis* (L.) L. (Rubiaceae), and *Salix tetrasperma* Roxb. (Salicaceae).

Terrestrial Vegetation

Mainland areas adjacent to the Mekong River and all the islands in the river have vegetation which is totally different from riverine facies. All terrestrial areas are flat and lack relief. Some larger islands have seasonal ponds, exposed bedrock, and narrow, shallow flood/rain runoff channels. Due to centuries of human abuse, the original (i.e. before humans arrived) vegetation now ranges from degraded to destroyed. There is no place in the study area that has not been disturbed by people with their associated settlements, cattle, annual fires, agriculture, and continuous logging. There are four basic forest types, none pristine, which often merge together.

Mixed evergreen + deciduous, seasonal, hardwood forest (mxf)

The original, pre-human impact, forest facies in much of the area was mxf, most of which has been obliterated or transformed into other facies. Only a few islands, e.g. Norong and Rongnieu, have vestiges of this kind of forest, which as the name indicates, is a mixture of evergreen + deciduous species [3,4,6]. The understory and ground flora are mostly more evergreen than in other forest types, while the trees, up to 25 m tall, are a mixture of evergreen and deciduous species.

Frequently seen herbs in mxf are: *Desmodium heterocarpon* (L.) DC. ssp. *angustifolium* Oha. (Leguminosae, Papilionoideae), *Justicia ventricosa* Wall. (Acanthaceae), *Calcareoboea bonii* (Pell.) Burtt. (Gesneriaceae)—all dicots; *Carex indica* L. var. *indica* (Cyperaceae), a monocot; and several Pteridophytes, viz. *Selaginella roxburghii* (Hk. & Grev.) Spring var. *roxburghii* (Selaginellaceae), and Polypodiaceae epiphytes *Drynaria quercifolia* (L.) J. Sm., *Pyrrosia lanceolata* (L.) Farw., and *P. stigmosa* (Sw.) Ching.

An understory of mostly evergreen shrubs and treelets, many spiny, consists of *Polyalthia evecta* (Pierre) Fin. & Gagnep. and *Desmos chinensis* L. (both Annonaceae), *Atalantia monophylla* (L.) DC. (Rutaceae), *Memecylon lilacinum* Zoll. & Mor. (Melastomataceae), *Ixora finlaysoniana* Wall.

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ex G. Don and *I. nigricans* R. Br. *ex* Wight & Arn. (Rubiaceae), and *Streblus asper* Lour. var. *asper* (Moraceae).

Evergreen trees, formerly common and now sparse and scattered, include: *Xylopia pierrei* Hance (Annonaceae), *Mammea siamensis* (Miq.) T. And. (Guttiferae, Photo 48), *Acronychia pedunculata* (L.) Miq. (Rutaceae), *Irvingia malayana* Oliv. *ex* Benn. (Irvingiaceae), *Lepisanthes tetraphylla* (Vahl) Radlk. (Sapindaceae), *Carallia brachiata* (Lour.) Merr. (Rhizophoraceae), *Eugenia fruticosa* (DC.) Roxb. and *E. grandis* Wight var. *grandis* (Myrtaceae), *Diospyros bejaudii* Lec. (Ebenaceae), *Chaetocarpus castanocarpus* (Roxb.) Thw., and *Drypetes roxburghii* (Wall.) Huru. (both Euphorbiaceae). Dicot woody climbers are frequent with: *Artabotrys hexapetalus* (L.f.) Bhar. (Annonaceae), *Celastrus paniculatus* Willd. (Celastraceae), *Tetrastigma harmandii* Pl. (Vitaceae), and *Dalbergia entadoides* Pierre *ex* Gagnep. (Leguminosae, Papilionoideae). The most obvious indicators of mxf are three species of *Calamus* (Palmae, rattans), viz. *C. rudentum* Lour., *C. siamensis* Becc. var. *siamensis* (the most common species), and *C. viminalis* Willd.

Bamboo + deciduous, seasonal, hardwood forest (bb/df)

This is the most prevalent and persistent forest type in the area. Severely degraded to destroyed mxf areas are replaced with bb/df, thus many forested areas are a mixture of declining mxf and rapidly developing bb/df—the absence of bamboo and lack of *Calamus* in bb/df being a good indicator of the actual forest facies. The bamboo component of bb/df consists almost entirely of *Bambusa bambos* (L.) Voss. *ex* Vilm. (Gramineae, Bambusoideae). This species, which is densely clumped, fire-resistant, and severely thorny, varies from dominating bb/df to absent, which depends on the extent of logging and fire on each island. In general, bb/df is more open, irregular and predominantly deciduous than mxf. Many bb/df areas include much secondary growth, thus there is great variation in the composition of bb/df on the islands.

The ground flora includes many annual and deciduous dicots and monocots, most of which flower and fruit during the rainy season. Typical annual dicots are: *Crotolaria acicularis* Ham. *ex* Bth., *C. montana* Hey. *ex* Roth, and *Mecopus nidulans* Benn. (all Leguminosae, Papilionoideae); *Borreria brachystema* (R. Br. *ex* Bth.) Val. and *Hedyotis verticillata* (L.) Lmk. (both Rubiaceae), *Lindernia ciliata* (Colsm.) Penn. and *Torenia violacea* (Aza. *ex* Blanco) Penn. (both Scrophulariaceae), *Dipteracanthus repens* (L.) Hassk. and *Justicia ventricosa* Wall. (both Acanthaceae).

Deciduous monocots are very diverse and provide most of the ground cover during the rainy season, which is best developed during July-September. Typical representatives are: *Murdannia edulis* (Stokes) Faden (Commelinaceae), *Halopegia brachystachys* Craib (Marantaceae), and Zingiberaceae with *Curcuma aurantiaca* van Zijp, *Globba schomburgkii* Hk. f. var. *schomburgkii*, and *Zingiber zerumbet* (L.) Sm. var. *zerumbet*. Orchidaceae are very prominent in bb/df with: *Brachycorythis helferi* (Rchb. f.) Summ., *B. laotica* (Gagnep.) Summ., *Habenaria lucida* Wall. e*x* Lindl., *Liparis rheedii* (Bl.) Lindl. and *L. siamensis* Rol. *ex* Dow., *Carex tricephala* Boeck. and *Fimbristylus dichotoma* (L.)

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Vahl ssp. *dichotoma* (both Cyperaceae) with *Aristida setacea* Retz., *Panicum notatum* Retz., and sometimes *Chrysopogon nemoralis* (Balan.) Holtt. (all Gramineae) also providing much cover.

Woody climbers in bb/df are all deciduous with: *Uvaria hahnii* (Fin. & Gagnep.) Sincl. (Annonaceae), *Capparis micracantha* DC. ssp. *micracantha* (Capparaceae), *Harrisonia perforata* (Blanco) Merr. (Simaroubaceae, Photo 7), *Calycopteris floribunda* (Roxb.) Lmk. and *Combretum latifolium* Bl. (both Combretaceae), *Ziziphus cambodiana* Pierre var. *cambodiana* and *Z. oenoplia* Mill. var. *oenoplia* (Rhamnaceae).

Trees in bb/df are mostly deciduous, the tallest ones being 20-25 m tall. Selected logging has resulted in significant decreases in many tall trees with valuable wood which has been used to build houses and boats. This extensive timber extraction has resulted in the extirpation of all tall trees on many islands and their depletion on a few islands where some of these trees still exist. The most exploited trees are *Dipterocarpus alatus* Roxb. *ex* G. Don and *Hopea odorata* Roxb. (both Dipterocarpaceae), *Xylia xylocarpa* (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) I. Niels (Leguminosae, Mimosoideae), *Sindora siamensis* Teysm. *ex* Miq. var. *siamensis* (Leguminosae, Caesalpinioideae), *Anogeissus acuminata* (Roxb. *ex* DC.) Guill. & Perr. and *Terminalia bellirica* (Gaertn.) Roxb. (both Combretaceae)—all deciduous, and *Irvingia malayana* Oliv. *ex* Benn. (Irvingiaceae), an evergreen species. As a result of the loss of forest integrity, erosion of organic material in the soil, fires, and depletion of wildlife, the forest facies has changed and is now dominated by trees which are not cut due to their inferior wood value, and most of which produce small, wind-dispersed seeds and do not require animals for distribution. *Lagerstroemia cochinchinensis* Pierre var. *ovalifolia* Furt. & Mont.—the most common component and *L. lecomtei* Gagnep. (Lythraceae), *Cratoxylum cochinchinense* (Lour.) Bl. and *C. formosum* (Jack) Dyer ssp. *pruniflorum* (Kurz) Gog. (Guttiferae), and *Terminalia triptera* Stapf (Combreataceae) are typical examples. *Canarium subulatum* Guill. (Burseraceae), *Schleichera oleosa* (Lour.) Oken (Sapindaceae), *Spondias pinnata* (L. f.) Kurz (Anacardiaceae), and *Vitex peduncularis* Wall. *ex* Schauer (Verbenaceae) are deciduous trees with animal-dispersed fruits that have not been extensively selected for logging. Many of these surviving trees have been damaged by fire or cutting and have coppicing trunks, irregular boles, and burned interiors. Annual fires during January-May, grazing, and continuous cutting of vegetation by encroachers have caused the elimination of seedlings and saplings of the tall, valuable tree species as well as deformed or otherwise damaged the growth of the remaining species.

Many secondary growth (sg) trees have become established in bb/df, especially with *Grewia eriocarpa* Juss. and *Microcos paniculata* L. (both Tiliaceae), *Markhamia stipulata* (Wall.) Seem. *ex* K. Sch. var. *stipulata* (Bignoniaceae), and *Trewia orientalis* (L.) Bl. (Ulmaceae). This aspect will be discussed in the section about secondary growth.

Deciduous, dipterocarp, seasonal, hardwood forest (ddf)

Throughout many areas in the lower elevations of northern Thailand and extending to the Seephandon wetlands, this kind of forest normally has an oak (Fagaceae) component, especially

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Quercus kerrii Craib [3,4,6]. No Fagaceae was found in the study area, although it is strongly suspected that this species of *Quercus* used to be there. This species is exploited for its hard wood, which makes an excellent charcoal and construction wood, as well as a source of tannins. The nuts (acorns) require animals for distribution, thus reestablishment of this species may also have been retarded by loss of wildlife throughout the region. This kind of forest is also known as savanna and is a fire-climax facies with a very distinct flora that is most extensive on the eastern mainland in the vicinity of Ou Chralang (village) as well as on Norong and Rongnieu islands. The general vegetation structure is open and single-storied, while in the rainy season the often dense ground flora 1-2 m tall is present (Photo 8). Typically bamboos are absent and most woody climbers are found on termite hills (termitaria). During the dry season the trees are leafless and the ground flora is bare and usually burned, exposing the poor, rocky soil. Ponds are scattered throughout ddf and are dry from November to June. Due to disturbance, ddf and bb/df often merge forming irregular boundaries which often contain a mixture of their respective species. In most instances the flora in bb/df and ddf is different.

The ground flora in ddf is mostly deciduous with a peak of development and flowering during July-September, providing a valuable habitat to many recently extirpated animal species. Domestic cows and water buffalo now roam freely in these places. As in bb/df the ground flora in ddf is very diverse and most luxurious in the rainy season, although the flora in these two kinds of forests is mostly different. Annual herbs include some common dicots, viz. *Salomonoia cantoniensis* Lour. (Polygalaceae), *Polycarpaea corymbosa* (L.) Lmk. (Caryophyllaceae), *Osbeckia setoso-annulata* Gedd. (Melastomataceae), and *Heliotropium strigosum* Willd. (Boraginaceae). Scrophulariaceae are very abundant with: *Lindernia spathacea* (Bon.) Bon., *L. viscosa* (Horn.) Bold., *Pierranthus capitatus* (Bon.) Bon., and *Pseudostriga cambodiana* Bon. Some annual monocots, also diverse, are: *Eriocaulon sexangulare* L. (Eriocaulaceae), *Murdannia gigantea* (Vahl) Bruck. (Commelinaceae); *Cyperus castaneus* Willd., *Fimbristylis adenolepus* Kern, *Liphocarpa microcephala* (R. Br.) Kunth and *L. hemisphaerica* (Roth) Goet.—all Cyperaceae. Gramineae compose the bulk of the ground flora and often form dense clusters. Examples of annual grasses are: *Andropogon chinensis* (Nees) Merr., *Capillipedium cinctum* (Steud.) A. Camus, *Enteropogon dolichostachya* (Lag.) Keng *ex* Laza., *Eragrostis bipinnata* (L.) Musc., *E. unioloides* (Retz.) Nees *ex* Steud., *Gymnopogon delicatulus* (Cl.) Bor, and *Microchloa indica* (L. f.) P. Beauv.

Deciduous dicot herbs are represented by *Eriosema chinense* Vogel (Leguminosae, Papilionoideae), *Knoxia brachycarpa* R. Br. *ex* Hk. f. (Rubiaceae), and *Euphorbia parviflora* L. (Euphorbiaceae). Deciduous monocots are far more abundant, with *Costus speciosus* (Koen.) J. E. Sm., *Curcuma gracillima* Gagnep., and *Kaempferia siamensis* Siri. (all Zingiberaceae, Photo 9); *Habenaria acuifera* Wall. *ex* Lindl., *H. mandersii* Coll. & Hemsl., and *H. rumphii* (Brogn.) Lindl. (Orchidaceae). Cyperaceae are well-represented with *Cyperus leucocephalus* Retz., *Rhynchospora rubra* (Lour.) Mak., and *R. longisetis* R. Br. Robust, deciduous Gramineae are the most conspicuous component of ddf ground flora. Some common examples are: *Aristida chinensis* Munro, *Capillipedium annamense* A. Camus, *C. assimile* (Steud.) A. Camus, *Chrysopogon nemoralis* (Balan.) Holtt., *Ischaemum indicum* (Houtt.) Merr., and *Polytoca digitata* (L. f.) Druce.

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Deciduous shrubs, scattered and mostly below 1 m tall, include: *Dillenia suffruticosa* (Griff.) Mart. (Dilleniaceae), *Ellipelopsis cherrevensis* (Pierre *ex* Fin. & Gagnep.) R. E. Fr. (Annonaceae), *Desmodium pulchellum* (L.) Bth. and *Lespedeza henryi* Schindl. (both Leguminosae, Papilionoideae), and *Bridelia harmandiana* Gagnep. (Euphorbiaceae).

The ddf also includes several, mostly evergreen, epiphytes, e.g. *Hoya diversifolia* Bl. and *H. kerrii* Craib (Asclepiadaceae), vines; *Dendrophthoe pentandra* (L.) Miq. and *D. curvata* (Bl.) Miq. (Loranthaceae, Photo 10), hemi-parasitic shrubs; and several Orchidaceae, *Cleisomeria pilosulum* (Gagnep.) Seid. & Garay being the only one found with flowers. *Clitoria mariana* L. (Leguminosae, Papilionoideae), *Thunbergia similis* Craib (Acanthaceae)—both dicots; and *Smilax verticalis* Gagnep. (Smilacaceae), a monocot, are the most common deciduous vines.

Seasonal ponds are scattered in ddf and have mostly annual, aquatic to amphibious herbs growing there. This will be discussed in the following section on ponds.

Trees in ddf are typically scattered, the species well-distributed, and almost all deciduous. Dipterocarpaceae are most abundant, hence the name for this forest type. The dominant dipterocarps are: *Dipterocarpus intricatus* Dyer (Photo 11) and *D. tuberculatus* Roxb. var. *tuberculatus*, *Shorea obtusa* Wall. *ex* Bl. and *S. siamensis* Miq. var. *siamensis.* Other common trees in ddf are: *Dillenia pentagyna* Roxb. (Dilleniaceae), *Bombax anceps* Pierre var. *anceps* (Bombacaceae), *Berrya mollis* Wall. *ex* Kurz Tiliaceae), *Buchanania glabra* Wall. *ex* Hk. f. and *B. lanzan* Spreng. (Anacardiaceae), *Pterocarpus macrocarpus* Kurz (Leguminosae, Papilionoideae), *Terminalia alata* Hey. *ex* Roth (Combretaceae), *Careya arborea* Roxb. (Lecythidaceae), *Mitragyna rotundifolia* (Roxb.) O.K. and *Morinda tomentosa* Hey. *ex* Roth (both Rubiaceae), *Diospyros ehretioides* Wall. *ex* G. Don (Ebenaceae), and *Aporosa octandra* (B.-H. *ex* D. Don) Vick. var. *yunnanensis* (Pax & Hoffm.) Schot (Euphorbiaceae).

Ponds

Shallow, rain-fed, ephemeral ponds are found scattered in all terrestrial forest types, especially ddf, during July to October (Photo 12). These habitats are totally dry during November to May. The amphibious to aquatic vegetation in ponds differs from riverine Zone 1 facies in being much more abundant, diverse, and with many more dicots. Almost all vascular plants found in ponds are rooted in mud, have an annual cycle from May to November, and include many more annuals than deciduous perennials.

Typical examples of dicots, all annuals, found in ponds are: *Nymphoides* (*Limnantherum tonkinense* Dop, Gentianaceae); many Scrophulariaceae, viz. *Dopatrium micrantha* (Bth.) Bth., *Lindernia cambodgiana* (Bon.) Phil. and *L. viatica* (Kerr *ex* Barn.) Phil. Annual monocots include Hydrocharitaceae with *Hydrilla verticillata* (L. f.) Roy., *Lagarosiphon roxburghii* Bth. and *Ottellia lanceolata* (Gagnep.) Dandy; *Sagittaria guaynensis* Humb. ssp. *lappula* (D. Don) Bogin and *S. trifolia* L. (Alismataceae, Photo 13), *Monochoria vaginalis* (Burm. f.) Presl (Pontederiaceae), and some *Typhonium flagelliforme* (Lodd.) Bl. (Araceae). Cyperaceae are well-represented with: *Cyperus*

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compactus Retz., *C. iria* L., *C. pilosus* Vahl; *Eleocharis acutangula* (Roxb.) Schult., *Fimbristylis miliacea* (L.) Vahl, and *F. tetragona* R. Br. *Echinochloa colona* (L.) Link (Gramineae) is also common.

No perennial dicots were found and only two deciduous, perennial monocots were seen, viz. *Cyperus brevifolius* (Rottb.) Hassk. (Cyperaceae) and *Ceratopteris thalictroides* (L.) Brongn. (Parkeriaceae, a pteridophyte).

Secondary growth (sg) and disturbed areas (da)

Because of extensive disturbance and destruction of the terrestrial vegetation, much of the initial (primary) vegetation in the study area has not regenerated. Secondary growth species have successfully invaded and matured in disturbed areas. For convenience, herbaceous plants, i.e. weeds, are included here since these plants are the initial colonisers of open land and are succeeded by woody species that are different from the plants they have replaced.

Many of the first herbaceous invaders found in gaps, clearings, or fields are the same as found on sandbars and beaches (riverine Zone 5), but in far more abundance and most being rapidly growing, annual herbs. Some of the more widespread dicot weeds are: *Mimosa diplotricha* C. Wright *ex* Sauv. var. *diplotricha* (a scrambling vine) and *M. pudica* L. (Leguminosae, Mimosoideae), *Ludwigia hyssopifolia* (G. Don) Exell (Onagraceae), *Mollugo pentaphylla* L. (Aizoaceae), *Ageratum conyzoides* L. and *Eupatorium odoratum* L. (both Compositae), *Heliotropium indicum* L. (Boraginaceae), *Solanum nigrum* L. (Solanaceae), *Alternanthera sessilis* L. var. *sessilis* (Amaranthaceae), *Phyllanthus amarus* Schum. & Thonn. and *Phyllanthus urinaria* L. (Euphorbiaceae). The most common monocot weeds are perennial Gramineae, viz. *Eleusine indica* (L.) Gaertn., *Imperata cylindrica* (L.) P. Beauv. var. *major (*Nees) C. E. Hubb. *ex* Hubb. & Vaugh., *Phragmites vallatoria* (Pluk. *ex* L.) Veld., and *Thysanolaena latifolia* (Roxb. *ex* Horn.) Honda—the latter three species being very robust and gregarious.

Woody secondary growth species are fast-growing, weak-wooded, and short-lived. Trees predominate many da/sg places with *Polyalthia cerasoides* (Roxb.) Bth. *ex* Bedd. (Annonaceae), *Grewia eriocarpa* Juss. and *Microcos paniculata* L. (both Tiliaceae), *Markhamia stipulata* (Wall.) Seem. *ex* K. Sch. var. *stipulata* (Bignoniaceae), *Antidesma ghaesembilla* Gaertn. (Euphorbiaceae), and *Trema orientalis* (L.) Bl. (Ulmaceae). *Harrisonia perforata* (Blanco) Merr. (Simaroubaceae), *Ziziphus cambodiana* Pierre var. *cambodiana* and *Z. oenoplia* Mill. var. *oenoplia* (Rhamnaceae)—all wickedly spiny; and *Anomianthus dulcis* (Dun.) Sincl. (Annonaceae) are common deciduous woody climbers present in degraded bb/df and da/sg.

Vegetation Map

A preliminary vegetation map of the Mekong River basin, Cambodia, was produced by the GIS section, WWF Cambodia in 2006. A satellite image map, Landsat7etm (February 2002) was used to

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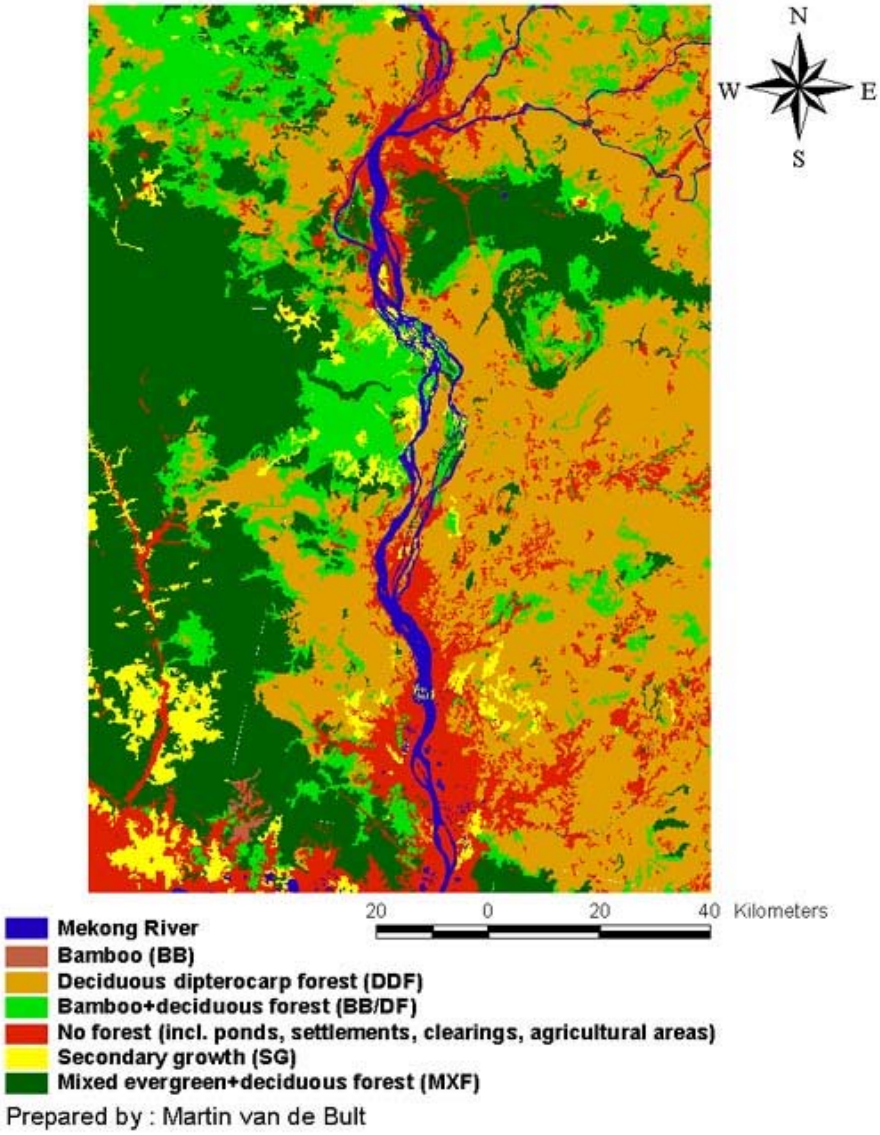
produce this map. A more accurate vegetation map of the study area (Map 3) was produced by Martin van de Bult after incorporating ground truthing data and GPS data points from all survey teams with information on vegetation types. Vegetation classification follows the terminology used in this report.



Map 3. Vegetation Map of the Mekong River, Cambodia

Rare Species

From this preliminary survey it is apparent that several species are rare to uncommon, some of them as result of exploitation (trees) and others naturally so. Rare trees include *Hopea odorata* Roxb. (Dipterocarpaceae), *Cynometra dongnaiensis* Pierre (Leguminosae, Caesalpinioideae), *Duabanga grandiflora* (Roxb. *ex* DC.) Walp. (Sonneratiaceae), *Pouteria obovata* (R. Br.) Baeh. (Sapotaceae)—

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all dicots, and *Caryota maxima* Bl. (Palmae), a monocot. *Brachystelma kerrii* Craib and *Ceropegia thorelii* Cost. (both Asclepiadaceae, Photo 14), *Aeginetia acaulis* (Roxb.) Walp. (Orobanchaceae), a leafless ground parasite; and *Burmannia wallichii* (Miers) Hk. f. (Burmanniaceae), a monocot and very delicate ground saprophyte; *Typhonium laoticum* Gagnep., in bb/df, and *T. flagelliforme* (Lodd.) Bl. (Araceae), in bb/df ponds are also rare. Several pteridophytes are also in this category with *Helminthostachys zeylanica* (L.) Hk. and *Ophioglossum petiolatum* Hk. (both Ophioglossaceae), terrestrial and deciduous; and *Platycerium wallichii* Hk. (Polypodiaceae), a massive evergreen epiphyte.

New Records

As far as it can be determined, 23 new records have been found for the Cambodian flora. Notes on distribution, forest type, and voucher specimens are provided.

1. *Acacia leucophloea* (Roxb.) Willd. (Leguiinsae, Mimosoideae); India, Burma, Thailand, southern Viet Nam, Java-Timor; bb/df; observed only
2. *Desmodium flexuosum* Wall. *ex* Bth. (Leguminosae, Papilionoideae); Burma, Thailand ddf; 06-874 (Photo 15)
3. *Indigofera zollingeriana* Miq. (Leguminosae, Papilionoideae); China, Taiwan, Laos, Viet Nam, Indonesia; da/sg; 07-123 (fruits)
4. *Rhodamnia cinerea* Jack var. *cinerea* (Myrtaceae); Thailand, Malay Peninsula, Borneo, Sumatra, Java; bb/df-mxf; 07-600
5. *Brachystelma kerrii* Craib (Asclepiadaceae); southern China, Thailand, Viet Nam; ddf; 07-5
6. *Diospyros oblonga* Wall. *ex* G. Don (Ebenaceae); India, Burma, Thailand, Malay Peninsula, Indonesia; bb/df; 07-598 (fruits)
7. *Ardisia attenuata* Wall. *ex* DC. (Myrsinaceae); China, Burma, Thailand, Viet Nam; mxf; Palee 1083 (Photo 16)
8. *Calcareoboea bonii* (Pell.) Burtt (Gesneriaceae); Thailand, Laos Viet Nam; mxf; 07-441
9. *Kaempferia siamensis* Siri. (Zingiberaceae); Thailand, ddf, 07-522
10. *Typhonium laoticum* Gagnep. (Araceae); Thailand, Laos; ponds in ddf; 07-483
11. *Brachycorythis helferi* (Rchb. f.) Summ. (Orchidaceae); Assam (E. India), Burma, Laos, Thailand; bb/df, 07-450
12. *Habenaria viridiflora* (Rottl. *ex* Sw.) R. Br. (Orchidaceae); Sri Lanka, India, Thailand; ddf, 07-607
13. *Liparis rheedii* (Bl.) Lindl. (Orchidaceae); Viet Nam, Thailand, Malay Peninsula, Sumatra; bb/df, 07-438
14. *Liparia siamensis* Rol. *ex* Dow. (Orchidaceae); Burma, Thailand, Laos; mxf, 07-440
15. *Nervilia punctata* (Bl.) Schltr. (Orchidaceae); Malay Peninsula, peninsular Thailand, Sumatra, Java; bb/df, 07-601 (leaves)
16. *Nervilia calcicola* Kerr (Orchidaceae); Malay Penisular, Thailand, Laos; bb/df, observed

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17. *Vandopsis gigantea* (Lindl.) Pfitz. (Orchidaceae); China, Laos, Thailand, Burma, Malay Peninsula; mxf, 07-155

18. *Fimbristylis brunneoides* Kern (Cyperaceae); Thailand, rv 2 & 3, 07-121

19. *Fimbristylis jucunda* (Cl.) Kern (Cyperaceae); Thailand, Laos, Viet Nam; rv 2 & 3, 07-122

20. *Murdannia discreta* (Craib) Thit. & Faden (Commelinaceae); northern Thailand; ddf; 07-417

21. *Amorphophallus koratensis* Gagnep. (Araceae); Thailand and Laos, bb/df; 07-145 (inflorescences), 07-425 (leaves)

22. *Cryptocoryne crispatula* Engl. var. *crispatula* (Araceae); Thailand and Laos, rv 2 & 3, 06-811

23. *Hemisorghum mekongense* (A. Camus) C.E. Hubb. *ex* Bor (Gramineae); Laos, Thailand, Burma; rv 5, 07-459; Photo 17

New Species

One new species, *Amorphophallus hemicryptus* Hett., (Araceae, Maxwell 06-896) was found on the west side of Kring Island on 16 November 2006 in bb/df (Photo 18). According to Dr. Wilbert Hetterscheid at Wageningen University, Netherlands, there are 8 species of *Amorphophallus* in Cambodia, *Amorphophallus hemicryptus* Hett. being the only one endemic to the country. Some of the unidentified species collected may perhaps be new, but taxonomic expertise for them is presently lacking.

Conservation

In recent years the islands in the study area have experienced an accelerated rate of encroachment and devastation by settlers moving into the region. All aspects of biodiversity have suffered because of these rampant, uncontrolled, and very destructive assaults on natural systems. Zones 1-5 of the riparian vegetation are not in any imminent danger, but Zone 6 and the terrestrial forests are in a deplorable condition and require immediate action to prevent further, essentially irreparable, degradation (Photos 19-26). Those islands which have been settled and/or deforested, e.g. Thaan, Khlee-ay, Dambong, Kondul, and Koh Tongdaeng can be sacrificed for settlement while several other islands, totally or in part, should be protected. Norong, Rongnieu, and Kring islands are recommended for protection because they have the most extensive and relatively intact forests. The ddf on Norong and Rongnieu islands should be protected since it is a vital habitat for wildlife as well as the most extensive and intact forest of this kind in the study area. Remnant mxf and bb/df areas on Kring, Norong, Rongnieu, and Veng Thom islands are also important since they still have viable populations of many plants which are now absent on other islands.

Being familiar with the ability of the Cambodian Government to implement official conservation action in the study area, it is suggested that some policies, which have become effective in northern Thailand, be considered for the Mekong River. A friendly relation must exist between

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conservation authorities and people on the islands. This can be initiated by having participatory discussions between the government representatives, WWF Cambodia, villagers and district leaders, school teachers, and interested outsiders. Discussions and training on the need for protection and effective conservation of all natural resources in the area should be explained, discussed, and agreed on along with official policies being implemented by the Cambodian Government. Migration, both seasonal and permanent, must be controlled since this is the basic reason that the area has been so severely degraded. Land and grazing rights, settlement locations and boundaries, as well as detailed regulations concerning land use must also be established. Some policies should be immediately implemented, viz. logging must be forbidden and all chain saws banned for use, hunting must stop, especially cutting trees to catch monkeys; burning forests must also cease. Islands which are presently settled and those which have been deforested should be replanted with indigenous vegetation. An important factor for both government officials and villagers to realise is that once the remaining forests disappear on the islands, it will take many decades (centuries?) for similar vegetation to develop. Recent destruction of the forest vegetation in many places is proof of this since the valuable trees that have been cut do not immediately reappear; da/sg comes first and the slow process of forest succession ensues. If people want to live on the islands they must not destroy the forest since if they do their future there will be short. Assistance in actually how to live in harmony with nature, modern agriculture methods which reduce soil erosion/degradation, prohibiting burning, and less destructive grazing by cattle can all be taught and practiced by the villagers. The problem with most local people is that they prefer not to abandon an established tradition, e.g. burning fields and exploiting all forest resources, until alternatives are proven to be better. This is exactly what must be done in the study area. Although strict conservation regulations are required, social contact must be made with villagers to convince them that destructive exploitation of the forest is not a sustainable way to live with presently very limited resources. If these people are concerned about their families in the future, they should be aware that continued devastation of the forests will certainly make life there more difficult to impossible in the near future. Dictatorial policies and militaristic enforcement, as in Thailand, are not recommended and will surely fail in Cambodia.

Future Botanical Work

This report can only be considered a preliminary study since not all the islands were surveyed throughout the year. A complete flora of the study area will require frequent and extensive collecting. Further collections, studies on forest dynamics, plant distributions, and observations on phenology will certainly add more vital information to the database as well as the vegetation map. Future reforestation and conservation will require more precise information of the location of seed sources, planting sites, and habitat requirements.

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Specimen Distribution

When possible, at least 4 specimens were collected of each species. One set was left at WWF Cambodia in Phnom Penh for donation to a future herbarium in Cambodia. CMU Herbarium maintains one set while duplicates will be sent to the National Herbarium Netherlands at Leiden and Harvard University Herbarium, USA. A complete set of all photographs taken are at CMU Herbarium and with Mr. Hourt at WWF Cambodia, Phom Penh.

Flora and Species Richness

A total of 683 species of vascular plants and 7 species of Bryophyta (mosses) were collected and recorded during the study (Table 1). The vascular flora and Bryophyta are also enumerated in an extensive database (Appendix 2). The database includes data on habit, habitat, abundance, elevation,life mode and leafing, flowering, and fruiting phenology.

Table 1. Summary of collecting and survey results

Division	Families	Species, ssp., var.
Angiospermae, Dicotyledonae	92	488
Angiospermae, Monocotyledonae	24	178
Pteridophyta	7	17
Bryophyta	7	7
Total	127	690

This report can only be considered a preliminary study since not all the islands were surveyed throughout the year. A complete flora of the study area will require frequent and extensive collecting. Further collection studies on forest dynamics, plant distributions, and observations on phenology will certainly add more vital information to the database as well as the vegetation map. Future reforestation and conservation will require more precise information of the location of seed sources, planting sites, and habitat requirements.

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P. Palee and M. van de Bult also helped in preparing the database, photography, GIS operation, computerised typing, and production of the report. Mr. M. Bezuijen, WWF Laos, is thanked for efficiently coordinating the project. Dr. E. L Webb (Asian Institute of Technology, Bangkok) kindly made some useful comments on the manuscript. Final corrections on the manuscript were added by my colleagues Dutsadee Nilubol and David Moore.

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Appendix 1: Photos

Photo 1. Many riverine areas do not have all zones clearly represented. This is a view of the SE side of Rongnieu Island during the hot, dry season where Zones 2-4 are absent. Zones 5 and 6 (beach and strand) as well as degraded terrestrial vegetation are distinct. 11 March 2007, photo P. Palee.

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Photo 2. Zones 1 and 2 at the height of the dry season with the lowest water level of the Mekong River, showing exposed rocks and terrestrial vegetation in the background. Real Island, 10 March 2007, photo P. Palee.

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Photo 3. Zone 2 showing *Telectadium edule* H. Baill. (Asclepiadaceae) clustered in rocky places. Norong Island, 17 March, photo P. Palee.

Photo 4. Zone 4 showing *Anogeissus rivularis* (Gagnep.) Lec. (Combretaceae) and *Acacia harmandiana* (Pierre) Gagnep. (Leguminosae, Mimosoideae) with canopies bent by the river current and trapping various debris (logs, bamboo, trash, *etc*.) when the water level is the highest. Kring Island, 1 August 2007, photo M. v.d. Bult.

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Photo 5. Zone 6 with dense adventitious roots of *Ficus virens* Ait. (Moraceae) which aid in soil stability. Svan Island, 1 August 2007, photo Eanghourt Khou.

Photo 6. *Pterospermum diversifolium* Bl. (Sterculiaceae), a common evergreen tree often found in Zone 6 produces large, woody, 5-angled (-valved) capsules with many winged seeds from October to March. Sompong Thom Island, 19 November 2006, photo P. Palee; Maxwell 06-927.

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Photo 7. *Harrisonia perforata* (Blanco) Merr. (Simaroubaceae) is a common, deciduous scandent-treelet species found in bb/df, da, and sg. It is easily recognized by the spine-tipped, mammiform tubercles covering the stems. Flowers are produced in May-June and fruits, which are eaten by animals, during June-August. Kring Island, photo M. v.d. Bult 937.

Photo 8. The general aspect of ddf is open, single-storied, with a dense 1-1.5 m tall herbaceous ground flora. Annual fire damage in the dry season has prevented woody species from regenerating. Rongnieu Island, 6 August 2007, photo Eanghourt Khou.

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Photo 9. *Kaempferia siamensis* Siri. (Zingiberaceae) is a common, deciduous, ground herb in ddf. The flowers have 3 sepals (not visible), 3 linear corolla lobes, 2 oblong staminodes, and a colourful, bifid lip. The paired leaves are flat on the ground. This is a new record for the Cambodian flora. Rongnieu Island, 7 August 2007, photo M. v.d. Bult, Maxwell 07-522.

Photo 10. *Dendrophthoe curvata* (Bl.) Miq. (Loranthaceae) is one of the three evergreen, epiphytic, hemiparasitic shrubs found on the islands. They infect various tree branches in all forests and produce berries eaten by birds. Rongnieu Island, 12 March 2007, photo P. Palee 1242.

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Photo 11. *Dipterocarpus intricatus* Dyer (Dipterocarpaceae), a common, deciduous tree in ddf, displays showy flowers during February-March and develops ripe 2-winged fruits in April along with new leaves. These fruits, with the bark of the tree in the background, are still immature. Rongnieu Island, 13 March 2007, photo Eanghourt Khou, Maxwell 07-75.

Photo 12. Seasonal ponds are common on many islands, especially in ddf. They are typically shallow, of various sizes, have mud substrate, and have water from about June to October. From November to May they are dry and lack vegetation. Rongnieu Island, 8 August 2007, photo M. v.d. Bult.

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Photo 13. *Sagittaria trifolia* L. (Alismataceae) is an uncommon, annual, aquatic herb found in ponds, on beaches and forests. One of the last flowering individuals was found in a nearly dry pond on Norong Island on 17 March 2007. Photo Eanghourt Khou, Palee 1249.

Photo 14. *Ceropegia thorelii* Cost. (Asclepiadaceae), a rare deciduous vine, is found in bb/df. Kring Island, 31 July 2007, photo Eanghourt Khou, Maxwell 07-444.

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Photo 15. *Desmodium flexuosum* Wall. *ex* Bth. (Leguminosae, Papilionoideae), the only vine in this genus found on the islands, is a rare deciduous species found in ddf and bb/df. It is a new record for the Cambodian flora. Rongnieu Island, 15 November 2006, photo P. Palee, Maxwell 06-874.

Photo 16. *Ardisia attenuata* Wall. *ex* DC. (Myrsinaceae), a new record for the Cambodian flora, is an evergreen treelet found in da and mxf. It produces fruits which people and animals eat. Kring Island, 11 November 2006, photo P. Palee 1083.

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Photo 17. *Heteropogon mekongense* (A. Camus) C.E. Hubb. *ex* Bor (Gramineae), a robust annual grass, is restricted to beaches (Zone 5). Ten days later the river water had submerged this plant. On a sandbar NW and opposite Svan Island, 1 August 2007, photo Eanghourt Khou; Maxwell 07-459. It is a new record for the Cambodian flora.

Photo 18. One confirmed new species, *Amorphophallus hemicryptus* Hett. (Araceae), was found during this project. It is a deciduous ground herb in bb/df which has the smallest inflorescence for the genus on the islands. Leaves are produced during the rainy season. Kring Island, 16 November 2006, photo P. Palee, Maxwell 06-896.

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Photo 19. Uncontrolled grazing, trampling of sensitive habitats, and fires, especially in the dry season, have caused extensive damage to the terrestrial vegetation. Kring Island, 30 July 2007, photo M.v.d. Bult.

Photo 20. The effects of unrestricted logging have been disastrous for biodiversity on the islands and adjacent mainland. Removal of trees vital for seed production and fires have effectively retarded or prevented regeneration of forests. The stump and boards are from *Dipterocarpus intricatus* Dyer (Dipterocarpaceae), an extensively exploited species in ddf. Norong Island, 17 March 2007, photo P. Palee.

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Photo 21. *Sindora siamensis* Teysm. *ex* Miq. var. *siamensis* (Leguminosae, Caesalpinioideae) cut by a logger the previous day (left) who is returning to his camp after another day of illegal handiwork (right). Norong Island, 16-17 March 2007, photo P. Palee.

Photo 22. As the most desired trees on the islands have been largely extirpated, logging of trees with lesser quality, such as *Lagerstroemia cochinchinensis* Pierre var. *ovalifolia* Furt. & Mont. (Lythraceae), is increasing. Removal of this tree will render bb/df even more degraded than it presently is. Norong Island, 2 August 2007, photo M. v.d. Bult (logging supervisor).

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Photo 23. Recently cut and soon to be burned land intended for rice cultivation at O Thmei (mainland); 17 March 2007, photo P. Palee.

Photo 24. As more people settle on the islands the integrity and quality of the vegetation declines. Scenes of recent destruction, such as this on the east side of Rongnieu Island, are increasing. Removal of strand vegetation and forest cover adjacent to it will result in the collapse of the land. 10 March 2007, photo P. Palee.

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Photo 25. Removal of stabilizing vegetation plus fires enable soil erosion to increase and island and mainland margins to collapse. This photo, from the north tip of Praeh-trabeik Island, shows how *Ficus benjamina* L. (Moraceae) and *Bambusa bambos* (L.) Voss. *ex* Vilm. (Gramineae, Bambusoideae) are unable to restrain extensive soil erosion due to the loss of forest integrity. These plants will eventually fall as the embankment continues to disappear. 14 November 2006, photo P. Palee.

Photo 26. Resin collection from *Dipterocarpus intricatus* Dyer (Dipterocarpaceae) is very destructive to the trees tapped plus the environment. Fire is used to stimulate resin flow, which is used for lighting, and often spreads to the forest. Trees damaged in this manner are frequently killed by infection or collapse. Rongnieu Island, 7 August 2007, photo M. v.d. Bult.

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Appendix 2

Database of vascular flora and Bryophyta resulting from this botanical survey

Key to abbreviations used in the database

	*	new record

Habit	cr	creeper
	h	herb
	l	treelet
	s	shrub
	sc	scandent
	t	tree
	v	vine
	wc	woody climber
Phenology	a	annual
	pe	perennial evergreen
	pd	perennial deciduous
Life mode	aqu	aquatic
	car	carnivorous
	cul	cultivated
	epi	epiphyte
	epl	epilithic
	gro	ground
	hyp	hyperparasite
	int	introduced, not native
	nat	naturalized
	par	parasite
	rhe	rheophyte
	sap	saprophyte
	str	“strangler”
	wee	weed

Month	ja	January
	fb	February
	mr	March
	ap	April
	my	May
	jn	June
	jl	July
	ag	August
	sp	September
	oc	October
	nv	November
	dc	December
Bedrock	ms	metamorphic sandstone
	sh	shale

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Abundance	0	probably extirpated
	1	down to a few individuals, in danger of extirpation
	2	rare
	3	medium abundance
	4	common, but not dominant
	5	abundant

Habitat	mxf	mixed evergreen + deciduous forest
	bb/df	deciduous forest with bamboo
	ddf	deciduous dipterocarp forest
	sg	secondary growth
	da	degraded areas
	rv 1	riverine zone 1, aquatic
	rv 2	riverine zone 2, rapids (“boong”)
	rv 3	riverine zone 3, “kai kum”
	rv 4	riverine zone 4, *Acacia-Anogeissus*
	rv 5	riverine zone 5, beach
	rv 6	riverine zone 6, strand

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Mekong River Cambodia database

Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Angiospermae, Dicotyledoneae													
Naravellia laurifolia Wall. *ex* Hk f. & Th.	Ranunculaceae	v	a	gro	2	bb/df	ms	25	30		oc-nv	my-dc	fruits
Dillenia ovata Wall. *ex* Hk. f. & Th.	Dilleniaceae	t	pd	gro	3	bb/df	ms	25	30	ja-fb	mr-ap	my-dc	
Dillenia parviflora Griff. var. *kerrii* (Criab) Hoogl.	Dilleniaceae	t	pd	gro	3	bb/df	ms	25	30	mr	ap	my-ja	flowers
Dillenia pentagyna Roxb.	Dilleniaceae	t	pd	gro	3	ddf	ms	25	30	fb-mr	mr-ap	my-nv	flowers
Dillenia suffruticosa (Griff.) Mart.	Dilleniaceae	s	pd	gro	3	ddf	ms	30	30	jl-ag		my-nv	flowers
Tetracera loureiri (Fin. & Gagnep.) Pierre *ex* Craib	Dilleniaceae	v	pe	gro	2	bb/df,rv 6	ms	25	30	ag-oc	ag-dc	ja-dc	fruits
Anomianthus dulcis (Dun.) Sincl.	Annonaceae	wc	pd	gro	3	bb/df,mxf,rv 6	ms	25	30	ap-my	jl-ag	ap-nv	fruits
Artabotrys hexapetalus (L.f.) Bhar.	Annonaceae	wc	pe	gro	3	mxf,ddf,sg	ms	25	30	fb-mr	mr-my	ja-dc	flowers,fruits
Desmos chinensis Lour.	Annonaceae	l	pe	gro	2	mxf	ms	25	30	ag-oc	nv-fb	ja-dc	fruits
Desmos velutinus (Hance) Ast	Annonaceae	l	pd	gro	2	bb/df,mxf	ms	25	30	ap-my	nv-dc	my-dc	fruits
Ellipelopsis cherrevensis (Pierre *ex* Fin. & Gagnep.) R.E. Fr.	Annonaceae	s	pd	gro	3	ddf	ms	30	30	jn-jl	ag-sp	my-nv	fruits
Goniothalamus marcanii Craib	Annonaceae	l	pe	gro	2	bb/df,mxf	ms	30	30	jl-ag	ag-sp	ja-dc	flowers
Melodorum fruticosum Lour.	Annonaceae	t	pe	gro	2	mxf	ms	25	30	mr-ap		ja-dc	flowers
Miliusa velutina (Dun.) Hk. f. & Th.	Annonaceae	t	pd	gro	3	ddf	ms	25	30	ap	jl	my-dc	
Polyalthia cerasoides (Roxb.) Bth. *ex* Bedd.	Annonaceae	t	pd	gro	3	ddf,bb/df	ms	25	30	ja-mr	mr-ap	mr-nv	flowers, fruits
Polyalthia evecta (Pierre) Fin. & Gagnep.	Annonaceae	t,l	pe	gro	3	mxf, rv 6	ms	25	30	oc-dc(mr)	oc-nv	ja-dc	flowers, fruits
Polyalthia modesta (Pierre) Fin. & Gagnep.	Annonaceae	s	pd	gro	3	rv 5-6	ms	20	25	dc	mr-ap	nv-jn	fruits
Polyalthia simiarum (Ham. *ex* Hk. f. & Th.) Bth. *ex* Hk. f. & Th.	Annonaceae	t	pe	gro	2	mxf	ms	25	30	fb-mr	jl	ja-dc	flowers
Polyalthia suberosa (Roxb.) Thw.	Annonaceae	t,l	pe	gro	2	mxf, rv 6	ms	25	30	oc-dc(mr)	oc-nv	ja-dc	flowers, fruits
Uvaria cordata (Dun.) Alst.	Annonaceae	wc	pe	gro	2	bb/df,da	ms	25	30	ag-oc	nv-fb	ja-dc	fruits
Uvaria hahnii (Fin. & Gagnep.) Sincl.	Annonaceae	wc	pd	gro	3	bb/df	ms	30	30	mr-ap	jl-ag	my-nv	fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Tiliacora triandra (Colebr.) Diels	Menispermaceae	v	pd	gro	3	da, sg	ms	25	30	jn-jl	ag-sp	my-ja	
Tinospora crispa (L.) Hk. f. & Th.	Menispermaceae	v	pd	gro	2	da	ms	25	30	fb-mr	my-jn	jn-ja	
Ceratophyllum demersum L.	Ceratophyllaceae	h	a	aqu	3	rv 1	ms	20	20	mr-my	ap-jn	nv-jn	
Rorippa indica (L.) Hiern	Cruciferae	h	a	gro	3	rv 5	ms	20	25	fb-mr	mr-ap	nv-jn	flowers
Capparis flavicans Kurz	Capparaceae	l,wc	pd	gro	2	bb/df	ms	30	30		ag-sp	my-dc	fruits
Capparis micracantha DC. ssp. *micracantha*	Capparaceae	wc	pd	gro	3	bb/df	sh, ms	25	30	sp-mr	ap-jn	my-fb	flowers
Cleome viscosa L.	Capparaceae	h	a	gro, wee	3	da, rv 5	ms	20	30	fb-ag	ap-ag	oc-sp	flowers, fruits
Crateva magna (Lour) DC.	Capparaceae	t,l	pd	gro	4	rv 2-6	ms	20	25	ag-nv(mr)	jl-ag	nv-oc	flowers
Stixis obtusifolia (Hk. f. & Th.) Pierre	Capparaceae	wc	pd	gro	2	da	ms	25	30	nv-mr	ja-ap	mr-ja	flowers
Scyphellandra pierrei Boiss.	Violaceae	s,l	pe	gro	2	bb/df	ms	25	30	oc-ja	dc-mr	ja-dc	flowers
Polygala chinensis L.	Polygalaceae	h	a	gro	2	ddf	ms	25	30	jl-sp	ag-oc	my-dc	flowers, fruits
Salomonoia cantoniensis Lour.	Polygalaceae	h	a	gro	3	ddf	ms	30	30	jn-sp	jl-oc	my-dc	flowers, fruits
Xanthophyllum lanceatum (Miq.) J. J. Sm.	Polygalaceae	t	pe	gro	2	rv 6	ms	20	25	fb-mr		ja-dc	flowers
Polycarpaea corymbosa (L.) Lmk.	Caryophyllaceae	h	a	gro	3	ddf	ms	25	30	sp-nv	nv-dc	my-dc	flowers
Portulaca oleracea L.	Portulacaceae	h	pe	gro,wee	3	da, rv 5	ms	25	30	oc-ja	ag-ja	ja-dc	flowers
Calophyllum sp.	Guttiferae	t	pe	gro	2	bb/df,mxf	ms	30	30			ja-dc	
Cratoxylum cochinchinense (Lour.) Bl.	Guttiferae	t	pd	gro	3	bb/df	ms	25	30	dc-ja	jl-ag	my-ja	fruits
Garcinia cowa Roxb.	Guttiferae	t	pe	gro	3	bb/df, mxf	ms	25	30	fb-ap (ag)	mr-my	ja-dc	♂
Garcinia sp.	Guttiferae	t	pe	gro	2	mxf	ms	25	30			ja-dc	
Mammea siamensis (Miq.) T. And.	Guttiferae	t	pe	gro	2	bb/df, mxf	ms	25	30	oc-dc	mr-ap	ja-dc	flowers, fruits
Casearia grewiifolia Vent. var. *grewiifolia*	Flacourtiaceae	l,t	pd	gro	3	bb/df,mxf,sg	ms	25	30	fb-mr	jl-ag	my-ja	flowers, fruits
Flacourtia indica (Burm. f.) Merr.	Flacourtiaceae	t	pd	gro	2	da,sg	ms	30	30	fb-ap	jl-sp	my-dc	
Homalium brevidens Gagnep.	Flacourtiaceae	t	pe	gro	3	rv 6	ms	25	30	jn-jl	sp-oc	ja-dc	flowers
Homalium caryophyllaceum (Zoll. & Mor.) Bth.	Flacourtiaceae	t	pe	gro	3	rv 6	ms	25	30	jl		ja-dc	flowers
Hydnocarpus anthelminthica Pierre *ex* Lanes.	Flacourtiaceae	t	pe	gro	3	rv 6, mxf	ms	25	30	nv-dc	ap-my	ja-dc	♀♂

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Dipterocarpus alatus Roxb. *ex* G. Don	Dipterocarpaceae	t	pd	gro	2	bb/df	ms	25	30	ja-fb	mr-ap	my-fb	
Dipterocarpus intricatus Dyer	Dipterocarpaceae	t	pd	gro	3	ddf	ms	25	30	fr-mr	ap	my-fb	flowers, imm. fruits
Dipterocarpus tuberculatus Roxb. var. *tuberculatus*	Dipterocarpaceae	t	pd	gro	3	ddf	ms	30	30	mr-ap	ap-my	ap-dc	
Hopea odorata Roxb.	Dipterocarpaceae	t	pd	gro	1	bb/df	ms	25	30	mr		my-fb	flowers
Shorea obtusa Wall. *ex* Bl.	Dipterocarpaceae	t	pd	gro	3	ddf	ms	25	30	mr-my	ap-jn	ap-fb	
Shorea roxburghii G. Don	Dipterocarpaceae	t	pd	gro	3	ddf	ms	25	30	ja-fb	mr-ap	mr-dc	fruits
Shorea siamensis Miq. var. *siamensis*	Dipterocarpaceae	t	pd	gro	3	ddf	ms	25	30	fb-mr	mr-ap	ap-dc	fruits
Ancistrocladus wallichii Pl.	Ancistrocladaceae	sc	pe	gro	2	streams,mxf	sh,ms	25	30	mr-my	jn-jl	ja-dc	flowers
Sida rhombifolia L. ssp. *rhombifolia*	Malvaceae	h	pe	gro,wee	3	da,sg	sh,ms	25	30	sp-mr	nv-ap	ja-dc	
Thespesia lampas (Cav.) Dalz. & Gibs. ssp. *lampas* var. *lampas*	Malvaceae	h	pd	gro	2	mxf,da	ms	25	30	sp-nv	nv-ja	my-dc	fruits
Urena lobata L. ssp. *lobata* var. *lobata*	Malvaceae	h	pe	gro, wee	3	da,rv 5	ms	25	30	sp-ja	oc-fb	ja-dc	
Bombax anceps Pierre var. *anceps*	Bombacaceae	t	pd	gro	3	ddf,bb/df	ms	25	30	ja-fb	mr	jn-dc	flowers,fruits
Ceiba pentandra (L.) Gaertn.	Bombacaceae	t	pd	gro, int, cul, nat	3	da	ms	30	30	ja-ap	my-jl	my-dc	
Byttneria echinata Wall. *ex* Kurz	Sterculiaceae	wc	pd	gro	3	wet areas in sg	ms	25	30	jn-jl	oc-dc	my-dc	fruits
Helicteres angustifolia L.	Sterculiaceae	s	pd	gro	3	ddf,bb/df	ms	25	30	jl-ag	nv-dc	my-dc	flowers
Helicteres elongata Wall. *ex* Boj.	Sterculiaceae	h	pd	gro	3	bb/df,da	ms	25	30	jl-dc	nv-dc	my-dc	flowers
Helicteres hirsuta Lour.	Sterculiaceae	s	pd	gro	3	bb/df,sg	ms	25	30	jl-dc	nv-fb	my-dc	flowers,fruits
Pterospermum cinnamomum Kurz	Sterculiaceae	t	pe	gro	3	mxf	ms	25	30	oc-ap	my-jn	ja-dc	
Pterospermum diversifolium Bl.	Sterculiaceae	t	pe	gro	3	rv 6,bb/df,mxf	ms	25	30	mr-ap (ag)	sp-nv	ja-dc	fruits
Sterculia balanghas L.	Sterculiaceae	t	pd	gro	3	ddf,bb/df	ms	25	30	mr-ap	oc-nv	ap-ja	
Sterculia foetida L.	Sterculiaceae	t	pd	gro	2	bb/df,mxf	ms	25	30		nv-dc	ap-dc	
Sterculia urena Roxb. var. *thorelii* (Pierre) Pheng.	Sterculiaceae	t	pd	gro	2	bb/df,ddf	ms	25	30	nv-dc	ja-mr	my-nv	flowers
Berrya mollis Wall. *ex* Kurz	Tiliaceae	t	pd	gro	3	ddf	ms	25	30	jn-jl	ag-nv	my-dc	fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Colona auriculata (Desf.) Craib	Tiliaceae	s	pd	gro	3	bb/df,da	ms	25	30	ag-nv	nv-ja	my-dc	flowers, fruits
Corchorus aestuans L.	Tiliaceae	h	a	gro	2	mxf,da	ms	25	30	ag-oc	nv-fb	my-dc	fruits
Grewia eriocarpa Juss.	Tiliaceae	t	pd	gro	3	bb/df,da,sg	ms	25	30	mr-ap	jl-sp	mr-nv	flowers
Grewia hirsuta Vahl	Tiliaceae	s	pd	gro	4	wet areas in sg	ms	25	30	jl-sp	oc-dc	my-fb	fruits
Microcos paniculata L.	Tiliaceae	t	pe	gro	4	bb/df,da/sg	ms	25	30	oc-nv	nv-ja	ja-dc	flowers,fruits
Microcos sinuata (Wall. *ex* Mast.) Burr.	Tiliaceae	l	pd	gro	2	rv 4	ms	20	25	mr-ap		nv-jn	flowers
Muntingia calabura L.	Tiliaceae	l	pe	gro,int,nat	2	da,sg	sh,ms	25	30	ja-dc	ja-dc	ja-dc	
Schoutenia ovata Korth.	Tiliaceae	t	pd	gro	3	rv 6, ddf	ms	25	30	jl	sp-nv	my-dc	flowers, fruits
Elaeocarpus sphaericus (Gaertn.) K. Sch.	Elaeocarpaceae	t	pd	gro	2	bb/df	ms	25	30	oc-nv	oc-nv	my-fb	flowers
Hiptage triacantha Pierre	Malpighiaceae	wc	pd	gro	2	rv 3-4	ms	20	25	jl-ag	oc-nv	my-nv	flowers
Biophytum reinwardtii (Zucc.) Klot.	Oxalidaceae	h	a	gro	2	ddf	ms	30	30	jl-sp	ag-oc	my-nv	
Biophytum sensitivum (L.) DC.	Oxalidaceae	h	a	gro	2	bb/df	ms	25	30	ag-oc	nv-ja	my-ja	flowers, fruits
Oxalis barrellieri L.	Oxalidaceae	h	a	gro,int,nat	2	bb/df,da	ms	30	30	jl-ag	ag-sp	my-nv	flowers, fruits
Acronychia pedunculata (L.) Miq.	Rutaceae	t	pe	gro	3	mxf	ms	25	30	jl-sp	nv-dc	ja-dc	flowers
Atalantia monophylla (L.) DC.	Rutaceae	l	pe	gro	2	mxf	sh,ms	25	30	oc-dc	my-jl	ja-dc	
Clausena excavata Burm. f. var. *excavata*	Rutaceae	l	pd	gro	3	ddf,bb/df,mxf	ms	25	30	fb-mr	jl-ag	fb-nv	flowers, fruits
Clausena wallichii Oliv. var. *wallichii*	Rutaceae	t	pd	gro	2	bb/df	ms	30	30		jl-ag	my-dc	fruits
Glycosmis pentaphylla (Retz.) DC. var. *pentaphylla*	Rutaceae	l,s	pe	gro	3	bb/df,mxf	ms	25	30	nv-dc	mr-ap	ja-dc	flowers,fruits
Murraya paniculata (L.) Jack	Rutaceae	l	pe	gro	2	mxf	ms	25	30	ap-my	sp-oc	ja-dc	
Paramignya scandens (Griff.) Craib var. *scandens*	Rutaceae	wc	pe	gro	2	mxf	ms	25	30	fb-mr	ag-nv	ja-dc	
Zanthoxylum rhetsa (Roxb.) DC.	Rutaceae	t	pd	gro	3	bb/df	ms	25	30	my-jn	sp-oc	my-dc	
Harrisonia perforata (Blanco) Merr.	Simaroubaceae	wc	pd	gro	3	bb/df,da,sg	ms	25	30	my-jn	jl-ag	my-fb	fruits
Quassia harmandiana (Pierre) Noot.	Simaroubaceae	t,l	pe	gro	3	rv 6, mxf	ms	25	30	ap-my	jn-ag	ja-dc	fruits
Irvingia malayana Oliv. *ex* Benn.	Irvingiaceae	t	pe	gro	2	bb/df,mxf	sh,ms	25	30	mr-my	jl	ja-dc	
Gomphia serrata (Gaertn.) Kanis	Ochnaceae	l	pe	gro	2	mxf	sh,ms	25	30	ja-mr	fb-mr	my-mr	flowers,fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Canarium subulatum Guill.	Burseraceae	t	pd	gro	3	bb/df	ms	30	30	mr-ap	jl-ag	my-dc	fruits
Aglaia odorata Lour.	Meliaceae	l	pe	gro	2	bb/df,mxf	ms	25	30	sp-nv	dc-fb	ja-dc	flowers
Azadiracta indica A. Juss.	Meliaceae	t	pd	gro	2	bb/df	ms	25	30	ja-fb	ap-my	mr-dc	imm. fruits
Chukrasia tabularis A. Juss.	Meliaceae	t	pd	gro	3	bb/df	ms	30	30	jl-ag	dc-ja	my-dc	
Walsura pinnata Hassk.	Meliaceae	l	pe	gro	2	bb/df,mxf	ms	25	30	nv-ja	nv-fb	ja-dc	flowers
Olax psittacorum (Willd.) Vahl	Olacaceae	wc	pe	gro	3	ddf	ms	30	30	ap-my	jl-ag	ja-dc	fruits
Celastrus paniculatus Willd.	Celastraceae	wc	pd	gro	3	ddf,bb/df	ms	30	30	mr-my	ag-sp	my-dc	fruits
Maytenus sp.	Celastraceae	l	pe	gro	2	bb/df,mxf	ms	25	30		oc-nv	ja-dc	fruits
Salacia macrophylla Bl.	Celastraceae	wc	pe	gro	3	mxf	ms	25	30	ja-ap	ap-my	ja-dc	flowers, imm. fruits
Siphonodon celastrineus Griff.	Celastraceae	t	pd	gro	2	bb/df	ms	30	30	ja-fb	dc-fb	ap-dc	
Colubrina pubescens Kurz	Rhamnaceae	s	pe	gro	3	bb/df,da	ms	25	30	sp-nv	nv-dc	ja-dc	flowers,fruits
Ventilago harmandiana Pierre	Rhamnaceae	wc	pd	gro	3	rv 6,mxf,bb/df	ms	25	30		jl-ag	my-mr	fruits
Ziziphus cambodiana Pierre var. *cambodiana*	Rhamnaceae	wc	pd	gro	3	bb/df	ms	25	30	ap-my	oc-dc	my-dc	fruits
Ziziphus oenoplia (L.) Mill. var. *oenoplia*	Rhamnaceae	sc	pd	gro	3	da,sg,ddf, bb/df,	ms	25	30	mr-ap	oc-dc	my-dc	flowers,fruits
Ampelocissus martinii Pl.	Vitaceae	wc	pd	gro	3	bb/df,da	ms	25	30	jl-ag	sp-oc	my-nv	flowers, fruits
Cayratia trifolia (L.) Dom. var. *trifolia*	Vitaceae	v	pe	gro	3	rv 6, bb/df,da	ms	25	30	ag-dc	jl-ja	ja-dc	flowers
Cissus modeccoides Pl. var. *modeccoides*	Vitaceae	v	a	gro	3	bb/df,da	ms	25	30	sp-oc	nv-dc	my-dc	fruits
Cissus quadrangularis L.	Vitaceae	v	pe	gro	2	bb/df,da	ms	25	30	nv-fb	dc-mr	ja-dc	flowers
Tetrastigma harmandii Pl.	Vitaceae	wc	pe	gro	3	mxf	ms	25	30	dc-mr	nv-ja	ja-dc	♀, fruits
Leea aequata L.	Leeaceae	h	pd	gro	3	ddf	ms	30	30	jl-ag	oc-nv	my-nv	flowers
Leea indica (Burm. f.) Merr.	Leeaceae	h/s	pe	gro	3	da,sg	ms	25	30	jl-oc	sp-nv	ja-dc	
Leea rubra Bl. *ex* Spreng.	Leeaceae	l	pd	gro	3	da,sg	ms	25	30	jl-ag	ag-oc	my-nv	flowers, fruits
Allophyllus cobbe (L.) Raeus.	Sapindaceae	t	pe	gro	3	da, sg	ms	25	30	jn-jl	jl-ag	ja-nv	fruits
Cardiospermum halicacabum L. var. *halicacabum*	Sapindaceae	v	a	gro,wee	3	rv 5, da	ms	20	25	fb-ag		ag-jn	flowers,fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Dimocarpus longan Lour. ssp. *longan* var. *longan*	Sapindaceae	t	pe	gro	3	bb/df	ms	25	30	mr-ap	ag-sp	ja-dc	fruits
Lepisanthes rubiginosa (Roxb.) Leenh.	Sapindaceae	l,t	ped	gro	3	bb/df,mxf	ms	25	30	fb-mr	mr-ap	mr-ja(dc)	flowers,fruits
Lepisanthes senegalensis (Poir.) Leenh.	Sapindaceae	l	ped	gro	3	bb/df,rv	ms	25	30	nv-mr	fb-ap	ja-dc	flowers,fruits
Lepisanthes tetraphylla (Vahl) Radlk.	Sapindaceae	t	pe	gro	3	mxf	ms	25	30	ja-mr	fb-mr	ja-dc	flowers,fruits
Schleichera oleosa (Lour.) Oken	Sapindaceae	t	pd	gro	3	bb/df	ms	25	30	fb-ap	jl	mr-dc	flowers, fruits
Buchanania glabra Wall. *ex* Hk f.	Anacardiaceae	l,t	pe	gro	3	ddf	ms	25	30	oc-ja	mr-ap	ja-dc	flowers,fruits
Buchanania lanzan Spreng.	Anacardiaceae	t	pd	gro	3	ddf	ms	25	30	ja-fb	mr-ap	mr-nv	fruits
Buchanania reticulata Hance	Anacardiaceae	t	pe	gro	3	bb/df	ms	25	30	oc-nv	mr-ap	ja-dc	flowers,fruits
Lannea coromandelica (Houtt.) Merr.	Anacardiaceae	t	pd	gro	3	ddf	ms	30	30	ja-mr	ap-my	ap-dc	
Mangifera camptosperea Pierre	Anacardiaceae	t	pe	gro	2	mxf	ms	25	30	ap-my	mr-ap	ja-dc	fruits
Semecarpus cochinchinensis Engl.	Anacardiaceae	t	pe	gro	2	mxf,bb/df	ms	25	30	dc-mr	mr-my	ja-dc	
Spondias pinnata (L. f.) Kurz	Anacardiaceae	t	pd	gro	3	bb/df	ms	25	30	ja-fb	dc-mr	my-ja	
Connarus cochinchinensis (Baill.) Pierre	Connaraceae	wc	pe	gro	2	mxf,da	ms	25	30	nv-mr	sp-oc	ja-dc	flowers,fruits
Acacia harmandiana (Pierre) Gagnep.	Leguminosae, Mimosoideae	t	pd	gro,epl	5	rv 4	ms	20	25	nv-dc	mr	oc-ag	flowers,fruits
**Acacia leucopholea* (Roxb.) Willd.	Leguminosae, Mimosoideae	t	pd	gro	2	bb/df	ms	25	30	ag-sp	ap-my	mr-nv	
Acacia pennata (L.) Willd. ssp. *kerrii* I. Niels.	Leguminosae, Mimosoideae	wc	pd	gro	3	da,sg	ms	25	30	fb-ag	sp-oc	mr-nv	flowers
Albizia lebbeckoides (DC.) Bth.	Leguminosae, Mimosoideae	t	pd	gro	3	streams,rv 6	ms	25	30	nv-dc	mr	my-dc	flowers,fruits
Entada rheedei Spreng.	Leguminosae, Mimosoideae	wc	pd	gro	3	bb/df,mxf	ms	25	30	mr-ap	oc-mr	mr-nv	flowers
Mimosa diplotricha C. Wright *ex* Sauv. var. *diplotricha*	Leguminosae, Mimosoideae	v	a	gro,int, nat, wee	3	da	ms	30	30	sp-nv	nv-ja	my-ja	
Mimosa pigra L.	Leguminosae, Mimosoideae	h	pe	gro,int, nat, wee	3	rv 5-6,da,sg	sh,ms	20	30	fb-ag	ja-sp	ja-dc	

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Mimosa pudica L.	Leguminosae, Mimosoideae	h	a	gro,int, nat, wee	3	da,sg	sh,ms	25	30	ag-mr	dc-ap	jl-ap	
Xylia xylocarpa (Roxb.) Taub. var. *kerrii* (Craib & Hutch.) I. Niels.	Leguminosae, Mimosoideae	t	pd	gro	1	bb/df	ms	25	30	ja-fb	oc-nv	my-dc	
*Bauhinia bracteata (*Grah. *ex* Bth.) Baker ssp. *bracteata*	Leguminosae, Caesalpinioideae	sc, wc	pe	gro	3	rv 5-6, bb/df, mxf	ms	25	30	ag-nv	jl-ag	ja-dc	flowers
Bauhinia championii (Bth.) Bth. var. *championii*	Leguminosae, Caesalpinioideae	wc	pd	gro	2	bb/df,mxf	ms	25	30	oc-nv		my-fb	flowers
Bauhinia racemosa Lmk.	Leguminosae, Caesalpinioideae	t	pd	gro	3	bb/df	ms	25	30	ag-oc	fb-mr	my-ja	fruits
Cassia fistula L.	Leguminosae, Caesalpinioideae	t	pd	gro	2	ddf,bb/df	sh,ms	25	30	fb-mr	nv-ja	my-ja	
Caesalpinia digyna Rottl.	Leguminosae, Caesalpinioideae	wc	pe	gro	3	bb/df	ms	25	30	jl-ag	fb-mr	ja-dc	flowers
*Caesalpinia mimosoides*Lmk.	Leguminosae, Caesalpinioideae	wc	pd	gro	3	da,sg	ms	25	30	oc-nv	fb-ap	my-dc	flowers
Crudia chrysantha (Pierre) K. Sch.	Leguminosae, Caesalpinioideae	t	pe	gro	3	rv 6	ms	25	30		jl-sp	ja-dc	fruits
Cynometra dongnaiensis Pierre	Leguminosae, Caesalpinioideae	t	pd	gro	1	bb/df	ms	25	30			my-dc	
Peltophorum pterocarpum (DC.) Back. *ex* K. Heyne	Leguminosae, Caesalpinioideae	t	pd	gro	2	mxf,ddf	ms	25	30	fb-mr	jn-jl	mr-dc	flowers
Senna tora (L.) Roxb.	Leguminosae, Caesalpinioideae	h	a	gro	2	ddf,bb/df,da	ms	25	30	sp-nv	nv-fb	my-dc	flowers,fruits
Sindora siamensis Teysm. *ex* Miq. var. *siamensis*	Leguminosae, Caesalpinioideae	t	pd	gro	2	bb/df,ddf	ms	25	30	ap-jn	ag-oc	mr-dc	
Aeschynomene americana L.	Leguminosae, Papilionoideae	h	a	gro,int, nat, wee	3	da	ms	25	30	sp-nv	dc-ja	jn-ja	flowers
Aganope thyrsiflora (Bth.) Polh.	Leguminosae, Papilionoideae	wc	pe	gro	3	bb/df	ms	30	30	jl-ag		ja-dc	flowers
Butea monosperma (Lmk.) Taub.	Leguminosae, Papilionoideae	t	pd	gro	3	da,sg,bb/df	ms	25	30	ja-fb	jn-jl	my-fb	

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Canavalia ensiformis (L.) A. DC.	Leguminosae, Papilionoideae	v	pd	gro	2	bb/df,da	ms	25	30	nv-dc	ja-mr	jn-mr	flowers
Centrosema pubescens Bth.	Leguminosae, Papilionoideae	v	a	gro	3	da,sg	ms	25	30	nv-ja	nv-ja	my-ja	flowers
Clitoria mariana L.	Leguminosae, Papilionoideae	v	pd	gro	3	ddf	ms	30	30	jl-ag	sp-oc	my-nv	flowers
Crotolaria acicularis Ham. *ex* Bth.	Leguminosae, Papilionoideae	h	a	gro	3	bb/df,da	ms	25	30	nv-fb	nv-fb	my-ja	flowers
Crotalaria bracteata Roxb. *ex* DC.	Leguminosae, Papilionoideae	h	a	gro	4	ddf,da	ms	25	30	oc-dc	oc-ja	my-ja	flowers
Crotalaria montana Hey. *ex* Roth	Leguminosae, Papilionoideae	h	a	gro	2	ddf	ms	30	30	jl-ag		my-nv	flowers
Crotalaria verrucosa L.	Leguminosae, Papilionoideae	h	a	gro	3	bb/df,da	ms	25	30	sp-nv	nv-ja	my-dc	flowers
Dalbergia cultrata Grah. *ex* Bth.	Leguminosae, Papilionoideae	t	pd	gro	3	ddf	ms	30	30	fb-mr	jn-jl	my-nv	
Dalbergia entadoides Pierre *ex* Gagnep.	Leguminosae, Papilionoideae	wc	pe	gro	3	mxf	ms	25	30	mr-ap		ja-dc	flowers
Dalbergia oliveri Gamb. *ex* Prain	Leguminosae, Papilionoideae	t	pd	gro	2	ddf	ms	25	30	jn-fb	fb-jn	my-dc	fruits
Dalbergia volubilis Roxb.	Leguminosae, Papilionoideae	wc	pd	gro	3	rv 4, 6	ms	20	25	mr-ap	jl-ag	mr-nv	flowers
Derris scandens (Roxb.) Bth.	Leguminosae, Papilionoideae	wc	pd	gro	3	rv 4, 6	ms	25	30	jl-sp	nv-dc	my-fb	flowers, fruits
Derris trifoliata Lour.	Leguminosae, Papilionoideae	wc	pe	gro	2	rv 6	ms	30	30	jl-ag		ja-dc	
Desmodium baccatum Schindl.	Leguminosae, Papilionoideae	l,s	pd	gro	3	bb/df,mxf	ms	25	30	oc-nv	dc-ja	ja-dc	flowers,fruits
**Desmodium flexuosum* Wall. *ex* Bth.	Leguminosae, Papilionoideae	v	pd	gro	2	ddf,bb/df	ms	25	30	sp-oc	nv-dc	ap-dc	fruits
Desmodium heterocarpon (L.) DC. ssp. *angustifolium* Oha.	Leguminosae, Papilionoideae	h	pd	gro	3	mxf,da	ms	25	30	nv-fb	nv-fb	jn-fb	flowers,fruits
Desmodium pulchelum (L.) Bth.	Leguminosae, Papilionoideae	s	pd	gro	3	ddf,bb/df	ms	25	30	ag-sp	nv-dc	my-dc	fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Desmodium triangulare (Retz.) Merr. ssp. *triangulare*	Leguminosae, Papilionoideae	h	pd	gro	3	bb/df	sh,ms	25	30	jl-nv	nv-ja	my-dc	flowers
Desmodium triflorum (L.) DC.	Leguminosae, Papilionoideae	h,cr	pe	gro,wee	3	ddf,da	ms	30	30	oc-ja	nv-fb	ja-dc	
Desmodium velutinum (Willd.) DC. ssp. *velutinum* var. *velutinum*	Leguminosae, Papilionoideae	h	pd	gro	3	mxf,da,sg	ms	25	30	oc-dc	nv-fb	my-dc	flowers
Eriosema chinense Vogel	Leguminosae, Papilionoideae	h	pd	gro	3	ddf	ms	30	30	jl-ag	sp-oc	my-nv	flowers, fruits
Flemingia strobilifera (L.) R. Br. *ex* Ait. f. var. *strolibifera*	Leguminosae, Papilionoideae	l,s	pd	gro	2	da,sg	ms	25	30	oc-nv	ja-fb	my-fb	
Indigofera cassioides Rottl. *ex* DC.	Leguminosae, Papilionoideae	s	pd	gro	3	ddf	ms	30	30	jl-ag	nv-dc	my-nv	flowers
Indigofera galegoides DC.	Leguminosae, Papilionoideae	s	pd	gro	2	bb/df	ms	25	30	sp-oc	nv-ja	my-dc	fruits
**Indigofera zollingeriana* Miq.	Leguminosae, Papilionoideae	t	pd	gro	2	da,sg	ms	25	30		ja-mr	mr-nv	fruits
Lespedeza henryi Schindl.	Leguminosae, Papilionoideae	l,s	pd	gro	3	ddf, bb/df	ms	25	30	ag-nv	nv-dc	my-dc	flowers
Mecopus nidulans Benn.	Leguminosae, Papilionoideae	h	a	gro	3	bb/df,da	ms	25	30	sp-nv	nv-dc	my-dc	flowers,imm. fruits
Mucuna pruriens (L.) DC. var. *pruriens*	Leguminosae, Papilionoideae	v	a	gro	2	bb/df,da	ms	25	30	oc-nv	fb-mr	my-dc	flowers
Paraderris elliptica (Wall.) Adema	Leguminosae, Papilionoideae	wc	pd	gro	3	rv 4, 6	ms	25	30	mr		mr-nv	flowers
Pterocarpus macrocarpus Kurz	Leguminosae, Papilionoideae	t	pd	gro	2	ddf,da	ms	25	30	jn-ag	sp-dc	my-dc	fruits
Rhynchosia bracteata Bth. *ex* Baker	Leguminosae, Papilionoideae	v	a	gro	3	da,sg	ms	25	30	nv-mr	dc-ap	nv-my	flowers,fruits
Spatholobus parviflorus (Roxb.) O.K.	Leguminosae, Papilionoideae	wc	pd	gro	3	ddf, bb/df	ms	25	30	jl-ag	nv-dc	my-ja	flowers
Teramnus labialis (L.f.) Spreng.	Leguminosae, Papilionoideae	v	a	gro	3	bb/df,da	ms	25	30	oc-nv	dc-ja	jn-ja	flowers
Uraria campanulata (Wall. *ex* DC.) Gagnep.	Leguminosae, Papilionoideae	h	pd	gro	3	bb/df,da	ms	25	30	sp-nv	nv-dc	my-dc	flowers,fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Uraria cordifolia Wall.	Leguminosae, Papilionoideae	h	pd	gro	2	ddf,bb/df	ms	25	30	oc-nv	nv-dc	my-dc	flowers,fruits
Uraria lagopodioides (L.) Desv. *ex* DC.	Leguminosae, Papilionoideae	h	pd	gro	3	ddf, bb/df	ms	25	30	ag-nv	nv-dc	my-dc	flowers,fruits
Uraria pierrei Schindl.	Leguminosae, Papilionoideae	h	pd	gro	3	ddf	ms	30	30	jn-ag	ag-oc	my-dc	flowers,fruits
Parinari anamensis Hance	Rosaceae	t	pe	gro	3	ddf,bb/df	ms	25	30	mr-ap	mr-my	ja-dc	flowers
Drosera burmannii Vahl	Droseraceae	h	a	gro	2	ddf	ms	30	30	ap-my	my-jn	jn-nv	
Drosera indica L.	Droseraceae	h	a	gro	2	ddf	ms	30	30	jn-ag	ag-oc	my-nv	flowers
Carallia brachiata (Lour.) Merr.	Rhizophoraceae	t	pe	gro	2	mxf	ms	25	30	dc-ja	my-jn	ja-dc	flowers
Anogeissus acuminata (Roxb. *ex* DC.) Guill. & Perr.	Combretaceae	t	pd	gro	3	bb/df	ms	25	30	oc-nv	nv-dc	my-dc	flowers,fruits
Anogeissus rivularis (Gagnep.) Lec.	Combretaceae	t	pd	gro,rhe	4	rv 4	sh,ms	20	25	jl-ag	sp	ag-jl	flowers
Calycopteris floribunda(Roxb.) Lmk.	Combretaceae	wc	pd	gro	4	bb/df	ms	25	30	ja-fb	mr-ap	mr-nv	fruits
Combretum latifolium Bl.	Combretaceae	wc	pd	gro	4	bb/df,mxf	sh,ms	25	30	dc-ja	mr	ap-dc	
Combretum quadrangulare Kurz	Combretaceae	t	pe	gro,rhe	2	wet areas in da, rv 6	ms	25	30	mr-my	oc-dc	ja-dc	flowers,fruits
Combretum trifoliatum Vent.	Combretaceae	sc	pd	gro,rhe	3	rv 5-6	ms	20	30	nv-mr	mr-ag	oc-jl	flowers,fruits
Terminalia alata Hey. *ex* Roth	Combretaceae	t	pd	gro	3	ddf	ms	25	30	my-jn	mr	my-dc	
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae	t	pd	gro	2	ddf,bb/df	ms	25	30	jl-ag	oc-dc	mr-dc	flowers
Terminalia chebula Retz. var. *chebula*	Combretaceae	t	pd	gro	3	ddf,bb/df	ms	25	30	mr-ap	nv-fb	mr-dc	
Terminalia mucronata Craib & Hutch.	Combretaceae	t	pd	gro	3	ddf	ms	30	30	ap	jl-sp	my-dc	
Terminalia triptera Stapf	Combretaceae	t	pd	gro	2	bb/df	ms	25	30	sp-oc	dc-ja	my-dc	flowers
Eugenia cumini (L.) Druce	Myrtaceae	t	pd	gro	3	ddf	ms	30	30	mr-ap	jl-ag	ap-dc	
Eugenia fruticosa (DC.) Roxb.	Myrtaceae	t	pe	gro	3	mxf	ms	25	30	mr-ap	jn	ja-dc	flowers
Eugenia grandis Wight var. *grandis*	Myrtaceae	t	pe	gro	2	mxf	ms	25	30	nv-mr	jl-ag	ja-dc	flowers
Eugenia grata Wight	Myrtaceae	t	pe	gro	2	mxf	sh,ms	25	30	ap-my	jl-ag	ja-dc	
Eugenia mekongensis Gagnep.	Myrtaceae	t	pd	gro,rhe	3	rv 3-6	ms	20	25	mr-ap	ap-my	nv-jn	flowers, imm. fruits
**Rhodamnia cinerea* Jack var. *cinerea*	Myrtaceae	l	pe	gro	2	bb/df, mxf	ms	25	30	my-jn	sp-oc	ja-dc	imm.fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Barringtonia acutangula (L.) Gaertn.	Lecythidaceae	t	pd	gro,rhe	3	rv 4-6	ms	20	25	ag-mr	oc-my	nv-jl	flowers,fruits
Careya arborea Roxb.	Lecythidaceae	t	pd	gro	3	ddf	ms	25	30	mr-ap	my-jn	my-fb	flowers
Memecylon caeruleum Jack	Melastomataceae	t,l	pe	gro	3	rv 6,bb/df,mxf	ms,sh	25	30	fb-mr	oc-dc	ja-dc	fruits
Memecylon lilacinum Zoll. & Mor.	Melastomataceae	t	pe	gro	3	mxf	ms	25	30	jl	oc-my	ja-dc	fruits
Memecylon scutellatum (Lour.) Naud.	Melastomataceae	l	pe	gro	3	ddf, streams in mxf	ms	25	30	ap-my	mr-ap	ja-dc	fruits
Memecylon umbellatum Burm. f.	Melastomataceae	t,l	pe	gro	3	rv 6,bb/df,mxf	ms	25	30	ja-fb	nv-dc	ja-dc	fruits
Osbeckia setoso-annulata Gedd.	Melastomataceae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Ammannia baccifera L.	Lythraceae	h	a	gro	3	wet areas in bb/df	ms	25	30	jl-sp	oc-nv	my-nv	fruits
Lagerstroemia cochinchinensis Pierre var. *ovalifolia* Furt. & Mont.	Lythraceae	t	pd	gro	4	bb/df	ms	25	30	jl-sp	fb-ap	my-dc	flowers
Lagerstroemia floribunda Jack var. *sublaevis* Craib	Lythraceae	t	pd	gro	3	bb/df	ms	25	30	sp-oc	oc-dc	my-dc	fruits
Lagerstroemia lecomtei Gagnep.	Lythraceae	t,l	pd	gro	4	bb/dfd, wet areas in sg	ms	25	30	jl-ag	nv-dc	my-ja	flowers, fruits
Lagerstroemia macrocarpa Kurz var. *macrocarpa*	Lythraceae	t	pd	gro	3	ddf,bb/df	ms	25	30	ap-my	jl-ag	my-dc	fruits
Lagerstroemia tomentosa Presl	Lythraceae	t	pd	gro	3	bb/df	ms	25	30	ap-my	ag-oc	my-nv	
Lagerstroemia villosa Wall. *ex* Kurz	Lythraceae	t	pd	gro	3	bb/df	ms	25	30	mr-my	ag-oc	my-nv	
Rotala indica (Willd.) Koeh.	Lythraceae	h	a	gro	3	wet areas in ddf	ms	25	30	jl-ag	oc-nv	my-dc	fruits
Duabanga grandiflora (Roxb. *ex* DC.) Walp.	Sonneratiaceae	t	pe	gro	1	mxf,da,sg	ms	30	30	ja-fb	ap-my	ja-dc	
Ludwigia hyssopifolia (G. Don) Exell	Onagraceae	h	a	gro,wee	3	rv 5, da	ms	20	25	ja-dc	ja-dc	ja-dc	flowers
Passiflora foetida L.	Passifloraceae	v	a	gro, int, nat, wee	3	da,sg	sh,ms	25	30	jl-mr	ag-ap	jl-my	
Coccinia grandis (L.) Voigt	Cucurbitaceae	v	a	gro	3	da,sg	ms	20	30	jl-mr	nv-mr	jl-ap	
Gymnopetalum integrifolium (Roxb.) Kurz var. *integrifolium*	Cucurbitaceae	v	a	gro	3	rv 5, da	ms	25	30	mr-ag	jn-oc	ja-oc	♂, fruits
Luffa cylindrica (L.) M. J. Roem.	Cucurbitaceae	v	a	gro	3	bb/df,da	ms	25	30	nv-mr	nv-ap	my-ap	flowers,fruits
Momordica charantina L.	Cucurbitaceae	v	a	gro,wee	3	da	ms	25	30	jn-oc	ag-nv	my-dc	

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Mukia maderaspatana (L.) M. J. Roem.	Cucurbitaceae	v	a	gro	2	bb/df,da	ms	25	30	oc-dc	oc-ja	my-dc	flowers,fruits
Scopella marginata (Bl.) Wilde & Duy. var. *marginata*	Cucurbitaceae	v	a	gro	2	bb/df,da	ms	30	30	jl-ag	ag-sp	my-nv	flowers, fruits
Solena heterophylla Lour. ssp. *heterophylla*	Cucurbitaceae	v	pd	gro	2	ddf	ms	30	30	jl-sp	sp-oc	my-dc	♂
Trichosanthes kirilowii Maxim.	Cucurbitaceae	v	a	gro	3	rv 5	ms	20	25			ja-jn	
Trichosanthes pubera Bl. ssp. *rubriflos* (Thor. *ex* Cay.) Duy. & Prue. var. *rubiflos*	Cucurbitaceae	v	a	gro	3	rv 6, da, sg	ms	25	30	jn-ag	jl-oc	my-dc	fruits
Zehneria marginata (Bl.) Kera.	Cucurbitaceae	v	a	gro	3	ddf,da	ms	25	30	ag-oc	nv-dc	my-dc	fruits
Tetrameles nudiflora R. Br. *ex* Benn.	Datiscaceae	t	pd	gro	3	bb/df	ms	25	30	mr-ap	ap-my	my-dc	
Glinus lotoides L.	Aizoaceae	h	a	gro	3	bb/df,da	ms	25	30	fb-ap	mr-my	nv-jn	flowers
Mollugo pentaphylla L.	Aizoaceae	h	a	gro,wee	3	bb/df,da	ms	25	30	nv-ag	ja-ag	sp-ap	flowers,fruits
Oenanthe javanica (Bl.) DC.	Umbelliferae	h	a	gro	3	rv 5	ms	20	25	mr-my	my-jn	nv-jn	flowers
Alangium salvifolium (L. f.) Wang. ssp. *hexapetalum* (Lmk.) Wang.	Alangiaceae	t	pd	gro	3	bb/df,da,sg	ms	25	30	ja-mr	ap-jn	ap-dc	fruits
Aphaenandra uniflora (Wall. *ex* G. Don) Brem.	Rubiaceae	h,cr	pd	gro	3	bb/df	ms	25	30	jl-ag	sp-oc	my-nv	flowers, imm. fruits
Borreria brachystema (R. Br. *ex* Bth.) Val.	Rubiaceae	h	a	gro	3	bb/df	ms	25	30	jl-ag	oc-nv	my-dc	fruits
Canthium berberidifolium Gedd.	Rubiaceae	l	pd	gro	2	ddf	ms	30	30		oc-nv	my-dc	
Catunaregam spathulifolia Tirv.	Rubiaceae	l	pd	gro	3	ddf,bb/df	ms	30	30	my-jn	sp-oc	my-dc	
Catunaregam tomentosum (Bl. *ex* DC.) Tirv.	Rubiaceae	l	pd	gro	3	ddf	ms	25	30	my-jn	jl-sp	my-dc	fruits
Dentella repens (L.) J. R. & G. Forst.	Rubiaceae	h	a	gro	3	rv 5	ms	20	25	ja-my	fb-jn	ja-jn	flowers
Fagerlindia (*Randia griffithii* Hk. f.)	Rubiaceae	sc	pe	gro	3	mxf,bb/df	ms	25	30		nv-dc	ja-dc	fruits
Gardenia cambodiana Pit.	Rubiaceae	l	pd	gro	2	bb/df	ms	30	30	my-jn	ag-sp	my-dc	imm. fruits
Haldina cordifolia (Roxb.) Rids.	Rubiaceae	t	pd	gro	3	bb/df	ms	25	30	ap-my	dc-df	my-dc	
Hedyotis chereevensis (Pierre *ex* Pit.) Fuku.	Rubiaceae	h	a	gro	3	rv 5-6	ms	25	30	jl-ag	ag-sp	ja-ag	flowers
Hedyotis kerwanhensis (Pierre *ex* Pit.) Maxw.	Rubiaceae	h	a	gro	3	bb/df,sg	ms	25	30	jl-ag	ag-nv	my-dc	flowers, fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Hedyotis nodiflora Wall. *ex* G. Don	Rubiaceae	t	pd	gro	2	ddf, bb/df	ms	25	30	jl-ag	oc-nv	my-dc	fruits
Hedyotis ovatifolia Cav.	Rubiaceae	h	a	gro	3	rv 5-6, ddf,bb/df	ms	25	30	jn-sp	ag-oc	ja-sp	flowers,fruits
Hedyotis pinifolia Wall. *ex* G. Don	Rubiaceae	h	a	gro	3	rv 5	ms	20	25	ja-mr	mr-ap	nv-jn	flowers
Hedyotis verticillata (L.) Lmk.	Rubiaceae	h	a	gro	3	wet areas in bb/df	ms	25	30	jl-ag	oc-nv	my-dc	flowers, fruits
Hymenodictyon orixense (Roxb.) Mabb.	Rubiaceae	t	pd	gro	3	bb/df	ms	25	30	jl-ag	oc-fb	my-dc	flowers
Ixora cibdela Craib	Rubiaceae	l	pe	gro	3	bb/df,mxf	sh,ms	25	30	ja-mr	mr-ap	ja-dc	fruits
Ixora finlaysoniana Wall. *ex* G. Don	Rubiaceae	l,s	pe	gro	3	bb/df,mxf	ms	25	30	mr-my	nv-dc	ja-dc	flowers,fruits
Ixora nigricans R. Br. *ex* Wight & Arn.	Rubiaceae	l	pe	gro	3	mxf	ms	25	30	fb-mr		ja-dc	flowers
Ixora sp.	Rubiaceae	l,s	pe	gro	3	bb/df,mxf	ms	25	30	ag-oc	nv-fb	ja-dc	fruits
Knoxia brachycarpa R. Br. *ex* Hk. f.	Rubiaceae	h	pd	gro	3	ddf	ms	25	30	jn-ag	jl-sp	my-nv	flowers,fruits
Mitragyna hirsuta Hav.	Rubiaceae	t	pd	gro	3	ddf	ms	30	30	jl-ag	fb-mr	my-fb	flowers
Mitragyna rotundifolia (Roxb.) O.K.	Rubiaceae	t	pd	gro	4	ddf,sg	ms	25	30	ap-my	sp-nv	my-ja	flowers, fruits
Morinda pandurifolia O. K. var. *oblonga* (Pit.) Craib	Rubiaceae	s,l	pd	gro,rhe	3	rv 3-5	sh,ms	20	30	nv-mr (my)	mr-ag	oc-my	flowers,fruits
Morinda tomentosa Hey. *ex* Roth	Rubiaceae	t	pd	gro	3	ddf	ms	25	30	mr-ap	jl-sp	mr-oc	flowers, fruits
Nauclea orientalis (L.) L.	Rubiaceae	t	pe	gro	3	rv 6	ms	25	30	mr-ap	jl-dc	ja-dc	fruits
Ophiorrhiza trichocarpon Bl. var. *trichocarpon*	Rubiaceae	h	pd	gro	3	bb/df	ms	30	30	jl-sp	sp-oc	my-nv	flowers
Oxyceros horrida Lour.	Rubiaceae	wc	pe	gro	3	bb/df	ms	25	30	ap-my	ag-sp	ja-dc	fruits
Psychotria montana Bl.	Rubiaceae	l	pe	gro	2	mxf	ms	25	30	ag-nv	nv-fb	ja-dc	fruits
Tamilnadia uliginosa (Retz.) Tirv. & Sastre	Rubiaceae	l	pd	gro	2	bb/df	ms	30	30	mr	jl-ag	my-dc	fruits
Xantonnea parviflora (O. K.) Craib var. *salicifolia* (Pierre *ex* Pit.) Craib	Rubiaceae	s	pd	gro,rhe	4	rv 2-5	ms	20	25	ja-my	ag	nv-jn	flowers, fruits
Ageratum conyzoides L.	Compositae	h	a	gro,nat, wee	4	rv 5, da,sg	sh,ms	20	30	jn-mr	ag-ap	oc-jn	
Blumea glandulosa DC.	Compositae	h	a	gro,wee	3	da,sg	ms	25	30	ja-mr	mr-ap	nv-jn	flowers
Blumea napifolia DC.	Compositae	h	a	gro,wee	3	da	sh,ms	25	30	ja-mr	mr-ap	nv-jn	flowers
Eclipta prostrata (L.) L.	Compositae	h	a	gro,wee	3	rv 5,da,sg	sh,ms	20	30	dc-mr	ja-ap	nv-jn	

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Elephantopus scaber L. ssp. *scaber* var. *scaber*	Compositae	h	pe	gro	3	bb/df,da	ms	25	30	oc-fb	oc-fb	ja-dc	flowers,fruits
Eupatorium odoratum L.	Compositae	h	pe	nat,wee	4	da,sg	sh,ms	25	30	ja-mr	mr-ap	ja-dc	
Grangea maderaspatana (L.) Poir.	Compositae	h	a	gro,wee	3	rv 5	ms	20	30	fb-ap	mr-my	nv-jn	flowers
Spilanthes paniculata Wall. *ex* DC.	Compositae	h	a	gro,wee	3	rv 5,da	ms	25	30	jn-sp	jl-oc	my-nv	flowers
Lobelia alsinoides Lmk.	Campanulaceae	h	a	gro	3	wet areas in ddf	ms	30	30	jn-ag	ag-sp	my-nv	flowers, fruits
Plumbago indica L.	Plumbaginaceae	h	pd	gro	2	bb/df	ms	25	30	oc-nv	ja-dc	my-dc	flowers
**Ardisia attenuata* Wall. *ex* DC.	Myrsinaceae	l	pe	gro	3	mxf,sg	ms	25	30	nv-ja	nv-fb	ja-dc	flowers,fruits
Ardisia villosa Roxb.	Myrsinaceae	l	pe	gro	2	mxf	ms	30	30	ap-jn	sp-nv	ja-dc	
Pouteria obovata (R. Br.) Baeh.	Sapotaceae	t	pd	gro	2	mxf	ms	25	30	mr-ap		mr-dc	
Diospyros bejaudii Lec.	Ebenaceae	t	pe	gro	3	bb/df	ms	30	30	jl-ag	jl-sp	ja-dc	fruits
Diospyros castanea (Craib) Flet.	Ebenaceae	t	pe	gro	2	ddf,sg	ms	30	30	mr-ap	jl-ag	ja-dc	fruits
Diospyros ehretoides Wall. *ex* G. Don	Ebenaceae	t	pd	gro	3	ddf	ms	25	30	mr-ap	oc-dc	my-dc	♂,fruits
**Diospyros filipendula* Pierre *ex* Pit.	Ebenaceae	t	pe	gro	2	ddf	ms	25	30		fb-ap	ja-dc	fruits
Diospyros malabarica (Desr.) Kostel. var. *siamensis* (Hochr.) Pheng.	Ebenaceae	t	pe	gro	3	rv 6, mxf	ms	25	30	mr-ap	oc-dc	ja-dc	♂,♀,fruits
Diospyros mollis Griff.	Ebenaceae	t	pd	gro	3	bb/df	ms	25	30	mr-ap	oc-dc	my-dc	♂,fruits
Diospyros montana Roxb.	Ebenaceae	t	pe	gro	3	rv 6	ms	25	30	mr-ap	jl-sp	ja-dc	fruits
**Diospyros oblonga* Wall. *ex* G. Don	Ebenaceae	t	pe	gro	2	bb/df	ms	30	30		jl-sp	ja-dc	fruits
Diospyros scalariformis Flet.	Ebenaceae	t	pe	gro	3	mxf	ms	25	30	mr-ap		ja-dc	♂
Diospyros venosa Wall. *ex* A. DC. var. *venosa*	Ebenaceae	t	pe	gro	3	bb/df,mxf	ms	25	30	fb-mr		ja-dc	♂
Jasminum siamensis Craib	Oleaceae	s	pd	gro	2	bb/df	ms	25	30	sp-nv	nv-ja	my-ja	fruits
Jasminum sp.	Oleaceae	wc	pd	gro	2	bb/df	ms	30	30	jl-ag		my-dc	
Myxopyrum smilacifolium (Wall.) Bl. ssp. *smilacifolium*	Oleaceae	wc	pe	gro	2	mxf	ms	30	30	fb-mr		ja-dc	flowers
Aganoneiron polymorphum Pierre *ex* Spire	Apocynaceae	v	pd	gro	2	da,sg	ms	25	30	jl-ag		my-nv	flowers
Aganosma marginata (Roxb.) DC.	Apocynaceae	wc	pd	gro	3	bb/df	ms	25	30	ap-my	dc-mr	my-fb	

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Holarrhena curtisii King & Gamb.	Apocynaceae	s	pd	gro	3	ddf	ms	30	30	my-ag	ag-oc	my-dc	flowers, fruits
Holarrhena pubescens Wall. *ex* G. Don	Apocynaceae	t	pd	gro	3	bb/df	ms	25	30	mr-my	ag-dc	mr-dc	flowers,fruits
Ichnocarpus frutescens (L.) W. T. Ait.	Apocynaceae	wc,v	pe	gro	2	mxf,da,sg	ms	25	30	nv-ja	dc-fb	ja-dc	flowers
Parameria laevigata (Juss.) Mold.	Apocynaceae	wc	pe	gro	3	bb/df,mxf	ms	25	30	oc-nv	fb-mr	ja-dc	flowers
Rauvolfia micrantha Hk. f.	Apocynaceae	h	pd	gro	2	bb/df	ms	30	30		jl-ag	my-dc	fruits
Wrightia arborea (Denn.) Mabb.	Apocynaceae	t	pd	gro	3	bb/df	ms	25	30	my-jn	jl-sp	my-dc	
**Brachystelma kerrii* Craib	Asclepiadaceae	h	pd	gro	2	ddf	ms	30	30	jl-ag		my-nv	flowers
Ceropegia thorelii Craib	Asclepiadaceae	v	pd	gro	2	bb/df	ms	30	30	jl-ag		my-nv	flowers
Hoya diversifolia Bl.	Asclepiadaceae	v	pe	epi	3	dof,bb/df	ms	25	30	mr-ap		ja-dc	flowers
Hoya kerrii Craib	Asclepiadaceae	v	pe	epi	2	ddf	ms	25	30	my-jl	jl-sp	ja-dc	
Hoya verticillata (Vahl) G. Don var. *verticillata*	Asclepiadaceae	v	pe	epi	2	ddf	ms	25	30	fb-mr	jn-ag	ja-dc	
Oxystelma esculentum (L. f.) R. Br.	Asclepiadaceae	v	pe	gro,rhe	3	rv 2-6	ms	20	30	ag-nv(mr)	ja-fb	ja-dc	flowers
Streptocaulon juventas (Lour.) Merr.	Asclepiadaceae	v	pe	gro	2	ddf, mxf,da	ms	25	30	ag-dc	sp-fb	ja-dc	flowers,fruits
Telectadium edule H. Baill.	Asclepiadaceae	sh	pd	epl	5	rv 2-3	ms	20	25	nv-dc(mr)	fb-mr	oc-ap	fruits
Toxocarpus villous (Bl.) Dcne.	Asclepiadaceae	v	pe	gro	2	mxf,da	ms	25	30	oc-dc	dc-fb	ja-dc	flowers
Tylophora harmandii Cost.	Asclepiadaceae	v	a	gro	2	bb/df,da	ms	25	30	sp-nv	nv-dc	my-dc	fruits
Zygostelma benthamii Baill.	Asclepiadaceae	v	pe	gro	2	bb/df	ms	30	30	oc-nv		ja-dc	
Mirteola petiolata (Gmel.) Torr. & A. Gray	Loganiaceae	h	a	gro	2	bb/df,da	ms	25	30	oc-dc	nv-ja	my-ja	flowers,fruits
Mitrasacme pygmaea R. Br. var. *pygmaea*	Loganiaceae	h	a	gro	3	ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Strychnos nux-vomica L.	Loganiaceae	t	pd	gro	3	ddf,bb/df	ms	25	30	mr-ap	dc-my	mr-ja	flowers
Strychnos rupicula Pierre *ex* Dop	Loganiaceae	wc	pe	gro	2	bb/df	ms	30	30		jl-ag	ja-dc	fruits
Canscora decussata (Roxb.) Schult.	Gentianaceae	h	a	gro	2	wet areas in ddf	ms	30	30	jn-ag	ag-sp	my-nv	flowers,fruits
Nymphoides (Limnanthemum tonkinense Dop)	Gentianaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers
Hydrolea zeylanica (L.) Vahl	Hydrophyllaceae	h	a	gro	3	ddf,bb/df	ms	25	30	sp-nv	nv-dc	my-dc	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Cordia dichotoma Forst. f.	Boraginaceae	t	pe	gro	3	rv 6	sh,ms	25	30	nv-dc(mr)	jl-nv	ja-dc	flowers,fruits
Heliotropium indicum L.	Boraginaceae	h	a	gro,wee	3	rv 5,da	ms	20	25	ja-dc	ja-dc	ja-dc	flowers
Heliotropium strigosum Willd.	Boraginaceae	h	a	gro	3	ddf	ms	30	30	jn-ag	jl-sp	my-dc	flowers, fruits
Rotula aquatica Lour.	Boraginaceae	s	pd	gro	3	rv 2-4	ms	25	30	fb-mr	mr-ap	nv-jl	flowers
Argyreia sp.	Convolvulaceae	v	pd	gro	2	ddf	ms	30	30	jn		my-dc	
Erycibe subspicata Wall. *ex* G. Don	Convolvulaceae	wc	pe	gro	3	rv 6	ms	25	30	ag-oc	nv-fb	ja-dc	fruits
Ipomoea mauritiana Jacq.	Convolvulaceae	v	a	gro	3	bb/df,da	ms	25	30	ag-sp	oc-nv	ja-dc	fruits
Jacquemontia paniculata (Burm. f.) Hall. f. var. *paniculata*	Convolvulaceae	v	a	gro	2	ddf,da	ms	25	30	nv-dc	ja-ap	jn-mr	flowers
Merremia hederacea (Burm. f.) Hall. f.	Convolvulaceae	v	a	gro	4	mxf.,sg	ms	25	30	oc-dc	oc-fb	jn-fb	flowers
Merremia hirta (L.) Merr. var. *hirta*	Convolvulaceae	v	a	gro	3	bb/df,da	ms	25	30	oc-dc	nv-fb	my-fb	flowers
Merremia vitifolia (Burm. f.) Hall. f.	Convolvulaceae	v	a	gro	3	da/sg	ms	25	30	ja-fb	mr-my	jn-dc	
Operculina turpethum (L.) S. Manso	Convolvulaceae	v	a	gro	3	rv 5,da,sg	ms	25	30	nv-dc	fb-mr	nv-jn	fruits
Capsicum annuum L.	Solanaceae	h	a	gro,int, nat,wee	3	da,sg	sh,ms	25	30	ja-dc	ja-dc	ja-dc	
Physalis angulata L.	Solanaceae	h	a	gro	3	rv 5, da	ms	20	25	ja-sp	ap-oc	nv-jn	flowers
Solanum nigrum L.	Solanaceae	h	a	gro,wee	3	rv 5, da	ms	20	30	nv-mr	dc-mr	oc-my	flowers,fruits
Solanum torvum Sw.	Solanaceae	h	a	gro, cul, int, nat	3	da, sg	ms	25	30	ag-ja	sp-mr	jn-ap	
Adenosma bracteosa Bon.	Scrophulariaceae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Dopatrium acutifolium Bon.	Scrophulariaceae	h	a	aqu, gro	2	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-dc	flowers
Limnophila laxa Bth.	Scrophulariaceae	h	a	gro	3	ddf	ms	25	30	oc-nv	dc-ja	my-dc	flowers
Limnophila micrantha (Bth.) Bth.	Scrophulariaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-dc	flowers
Limnophila repens (Bth.) Bth.	Scrophulariaceae	h	a	gro	3	wet areas in ddf	ms	25	30	sp-nv	nv-dc	my-dc	flowers
Lindenbergia muraria (Roxb. *ex* D. Don) R. Br.	Scrophulariaceae	h	a	gro	3	rv 5,da	ms	20	25	fb-mr	mr-ap	oc-jn	flowers
Lindenbergia philippensis (Cham.) Bth.	Scrophulariaceae	h	a	gro	3	rv 5,da	ms	20	25	mr-ag	mr-ag	oc-jn	flowers
Lindernia antipoda (L.) Alst.	Scrophulariaceae	h	a	gro,wee	3	rv 5,da	ms	20	30	ja-sp	mr-oc	nv-jn	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Lindernia cambodgiana (Bon.) Phil.	Scrophulariaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, imm. fruits
Lindernia ciliata (Colsm.) Penn.	Scrophulariaceae	h	a	gro	3	ddf	ms	30	30	jl-ag	ag-oc	my-nv	flowers, fruits
Lindernia crustacea (L.) F. Muell. var. *crustacea*	Scrophulariaceae	h	a	gro,wee	3	rv 5, da	ms	20	30	ja-sp	mr-oc	nv-jn	flowers
Lindernia spathacea (Bon.) Bon.	Scrophulariaceae	h	a	gro	3	ddf	ms	25	30	oc-nv	dc-ja	my-dc	flowers
Lindernia viatica (Kerr *ex* Barn.) Phil.	Scrophulariaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers
Lindernia viscosa (Horn.) Bold.	Scrophulariaceae	h	a	gro	3	wet areas in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers
Pierranthus capitatus (Bon.) Bon.	Scrophulariaceae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	jn-dc	flowers
Pseudostriga cambodiana Bon.	Scrophulariaceae	h	a	gro	3	ddf	ms	30	30	jl-ag	sp-oc	my-nv	flowers
Scoparia dulcis L.	Scrophulariaceae	h	a	gro,nat, wee	3	rv 5, da	ms	25	30	mr-ag	ap-sp	oc-jn	flowers
Striga asiatica Lour.	Scrophulariaceae	h	a	gro	2	ddf	ms	25	30	jn-ag	ag-sp	my-nv	flowers, fruits
Torenia flava B.-H. *ex* Bth.	Scrophulariaceae	h	a	gro	3	bb/df	ms	30	30	jl-sp	ag-oc	my-dc	flowers, fruits
Torenia laotica Bon.	Scrophulariaceae	h	a	gro	3	bb/df	ms	30	30	jn-ag	jl-sp	my-nv	flowers
Torenia thorelii Bon.	Scrophulariaceae	h	a	gro	3	bb/df,da	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Torenia violacea (Aza. *ex* Blanco) Penn.	Scrophulariaceae	h	a	gro	3	bb/df,da	ms	25	30	jl-mr	nv-fb	my-mr	flowers,fruits
Verbascum chinense (L.) Sant.	Scrophulariaceae	h	a	gro	3	rv 5	ms	20	25	fb-mr	mr-ap	nv-jn	flowers,fruits
Aeginetia acaulis (Roxb.) Walp.	Orobanchaeae	h	pd	gro,par	2	ddf	ms	30	30	jl-ag		leafless	flowers
Aeginetia indica Roxb.	Orobanchaeae	h	pd	gro,par	2	bb/df	ms	25	30	jl-sp	sp-oc	leafless	flowers
Utricularia bifida L.	Lentibulariaceae	h	a	aqu, gro	2	ponds in ddf	ms	30	30	jl-sp	ag-oc	jl-nv	
Utricularia pierrei Pell.	Lentibulariaceae	h	a	gro	2	wet areas in ddf	ms	30	30	jl-ag		my-oc	flowers
Utricularia striatula Sm.	Lentibulariaceae	h	a	gro	2	wet areas in ddf	ms	25	30	ag-nv	nv-dc	jl-dc	flowers
**Calcareoboea bonii* (Pell.) Burtt	Gesneriaceae	h	pd	gro	3	bb/df, mxf	ms	30	30	jl-ag	sp-oc	my-nv	flowers, imm. fruits
Markhamia stipulata (Wall.) Seem. *ex* K. Sch. var. *stipulata*	Bignoniaceae	t	pd	gro	3	da,sg	ms	25	30	nv-ag	sp-ap	my-ja	
Millingtonia hortensis L. f.	Bignoniaceae	t	pd	gro	3	bb/df	ms	25	30	ap-sp	oc-nv	my-oc	

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Oroxylum indicum (L.) Kurz	Bignoniaceae	t	pd	gro	2	da, sg	ms	25	30	jn-jl	jl-ag	my-dc	
Stereospermum cylindricum Pierre *ex* Dop	Bignoniaceae	t	pd	gro	3	bb/df	ms	30	30	jl-ag	sp-oc	my-dc	flowers, imm. fruits
Barleria strigosa Willd.	Acanthaceae	h	pd	gro	3	ddf,da	ms	25	30	jl-oc	nv-fb	jn-ja	flowers, fruits
Dipteracanthus repens (L.) Hassk.	Acanthaceae	h	a	gro	3	bb/df	ms	25	30	jl-nv	fb	my-fb	flowers,fruits
Dyschoriste depressa Nees	Acanthaceae	h	a	gro	3	bb/df	ms	25	30	nv-dc	nv-fb	my-fb	flowers
Hemigraphis modesta R. Ben.	Acanthaceae	h	pd	gro	2	rv 4,6	ms	20	25	fb-mr	ap-my	nv-jn	flowers
Hydrophila phlomoides Nees	Acanthaceae	h	a	gro	3	wet areas in ddf,bb/df	ms	25	30	oc-nv	dc-mr	my-mr	flowers,fruits
Justicia ventricosa Wall.	Acanthaceae	h	pe	gro	3	bb/df,mxf	ms	25	30	nv-dc		ja-dc	flowers
Justicia sp.	Acanthaceae	h	a	gro	2	wet areas in ddf	ms	30	30	jl-ag		my-nv	flowers
Lepidagathis incurva Ham. *ex* D. Don	Acanthaceae	h	pe	gro	3	bb/df	sh,ms	25	30	oc-mr	dc-ap	ja-dc	flowers
Nelsonia canescens (Lmk.) Spreng.	Acanthaceae	h	pe	gro	3	bb/df,da	ms	25	30	ja-mr	mr-ap	ja-dc	flowers
Neuracanthus tetragonostachyus Nees ssp. *tetragonostachyus*	Acanthaceae	a	a	gro	3	bb/df	ms	25	30	oc-nv	dc-ja	jn-ja	flowers
Peristrophe acuminata Nees	Acanthaceae	h	pe	gro	3	bb/df	ms	25	30	oc-nv(mr)	dc-ja	ja-dc	flowers
Pseuderanthemum poilanei R. Ben.	Acanthaceae	h	pe	gro	3	bb/df,mxf	ms	25	30	oc-nv	dc-ja	ja-dc	flowers
Ptyssiglotis kunthiana (Nees) B. Han.	Acanthaceae	h	a	gro	3	bb/df	ms	25	30	oc-dc	fb-mr	my-mr	flowers,fruits
Rungia parviflora (Retz.) Nees var. *parviflora*	Acanthaceae	h	a	gro	3	bb/df	ms	25	30	sp-nv	nv-dc	my-dc	flowers
Strobilanthes schomburgkii (Craib) J. R. I. Wood	Acanthaceae	h	a	gro	3	bb/df	ms	30	30	ja		jn-fb	
Thunbergia similis Craib	Acanthaceae	v	pd	gro	3	ddf	ms	25	30	jl-sp	oc-nv	my-nv	flowers
Clerodendrum godefroyi O. K.	Verbenaceae	l	pe	gro	2	bb/df,mxf	ms	25	30	oc-nv	dc-ja	ja-dc	flowers
Clerodendrum paniculatum L.	Verbenaceae	l,h	pd	gro	2	bb/df	ms	30	30	ag-oc	nv-dc	my-dc	
Clerodendrum serratum (L.) Moon var. *wallichii* Cl.	Verbenaceae	h	pd	gro	2	ddf	ms	30	30	jl-sp	sp-oc	my-dc	flowers, imm. fruits
Congea tomentosa Roxb. var. *tomentosa*	Verbenaceae	wc	pd	gro	3	bb/df	ms	25	30	fb-ap	ap-jn	ap-fb	
Glossocarya siamensis Craib	Verbenaceae	wc	pe	gro	3	rv 6	ms	25	30	jl-ag	oc-nv	ja-dc	flowers

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Gmelina philippensis Cham.	Verbenaceae	sc	pd	gro	2	bb/df, da	ms	25	30	ag-fb	nv-fb	my-fb	flowers,fruits
Paravitex sp.	Verbenaceae	s	pd	gro,rhe	3	rv 3-5	ms	20	25	mr-ap(ag)	ap-jl	oc-jn	flowers,fruits
Phyla nodiflora (L.) Greene	Verbenaceae	h	a	gro	3	rv 5	ms	20	25	mr-ag	ap-sp	oc-jl	flowers
Premna coriacea Cl. var. *coriacea*	Verbenaceae	wc	pe	gro	2	rv 6	ms	25	30	jl-ag	sp	ja-dc	flowers
Premna nana Coll. & Hemsl.	Verbenaceae	l,h	pd	gro	2	ddf	ms	30	30	ap-my	jl-ag	my-dc	fruits
Vitex limoniifolia Wall. *ex* Kurz	Verbenaceae	t	pd	gro	3	ddf,bb/df	ms	25	30	jl-sp	oc-dc	my-dc	
Vitex peduncularis Wall. *ex* Schauer	Verbenaceae	t	pd	gro	3	ddf,bb/df	ms	25	30	jl-ag	my-jl	ap-dc	flowers
Hyptis brevipes Poir.	Labiatae	h	a	gro	3	rv 5,da,sg	ms	25	30	jl-sp	sp-oc	my-nv	flowers, fruits
Leonotis nepetaefolia (L.) R. Br.	Labiatae	h	a	gro,wee,nat	3	rv 5, da	ms	25	30	ja-mr	mr-ap	nv-jn	flowers,fruits
Leucas decemdentata (Willd.) J. Sm.	Labiatae	h	a	gro,wee	3	bb/df,da	ms	25	30	sp-mr	nv-ap	my-ap	flowers
Orthosiphon spiralis (Lour.) Merr.	Labiatae	h	a	gro	2	bb/df	ms	30	30	jl-ag	ag-sp	my-dc	flowers,fruits
Platostoma hispidum (L.) Pat.	Labiatae	h	a	gro	2	ddf,bb/df	ms	25	30	oc-nv	dc-ja	my-dc	flowers
Chenopodium ficifolium Sm.	Chenopodiaceae	h	a	gro,wee	3	rv 5	ms	20	25	mr-my	ap-jn	nv-jn	
Alternanthera sessilis (L.) DC. var. *sessilis*	Amaranthaceae	h	a	gro,wee	2	bb/df,da	ms	25	30	jl-dc	ag-fb	jn-fb	flowers
Amaranthus spinosus L.	Amaranthaceae	h	a	gro, wee	3	rv 5, da	ms	25	30	my-nv	jn-dc	my-dc	
Celosia argentea L.	Amaranthaceae	h	a	gro, wee	3	rv 5, da	ms	25	30	jn-sp	jl-oc	my-nv	flowers, fruits
Psilotrichum ferrugineum (Roxb.) Moq.-Tand.	Amaranthaceae	h	a	gro	3	rv 5,ddf,bb/df	ms	25	30	jl-nv	sp-dc	my-dc	flowers
Polygonum plebium R. Br.	Polygonaceae	h	a	gro,wee	3	rv 5, da	ms	20	30	dc-ap	ja-my	nv-jn	flowers
Polygonum pubescens Bl.	Polygonaceae	h	a	gro	3	rv 3-5, streams, wet areas	ms	20	25	dc-mr	ja-ap	nv-jn	
Dalzellia carinata (Lec.) C. Cuss.	Tristichaceae	h	pd	aqu,epl, rhe	3	rv 2	ms	20	20	fb-mr	mr	mr-my	flowers
Piper retrofractum Vahl	Piperaceae	v	pe	gro	2	bb/df	ms	25	30	my-jn	nv-dc	ja-dc	fruits
Beilschmiedia aff. glomerata Elm.	Lauraceae	t	pe	gro	3	rv 6	ms	25	30		jl-ag	ja-dc	fruits
Cryptocarya oblongifolia Bl.	Lauraceae	t	pe	gro	3	bb/df,mxf	ms	25	30	jn	nv-mr	ja-dc	fruits
Litsea glutinosa (Lour.) C.B. Rob. var. *glutinosa*	Lauraceae	t	pd	gro	3	bb/df	ms	25	30	ag-sp	jl-ag	my-ja	fruits

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Illigera thorelii Gagnep.	Hernandiaceae	wc	pd	gro	3	bb/df,da	ms	25	30	sp-oc	ja-fb	my-fb	imm. fruits
Dendrophthoe curvata (Bl.) Miq.	Loranthaceae	s	pe	epi,par	3	ddf,mxf	ms	25	30	jl-ap	oc-my	ja-dc	flowers
Dendrophthoe pentandra (L.) Miq.	Loranthaceae	s	pe	epi,par	3	ddf	ms	25	30	mr-ap	mr-my	ja-dc	flowers
Macrosolen cochinchinensis (Lour.) Tiegh.	Loranthaceae	s	pe	epi,par	4	rv 4	ms	20	30	mr-ap	my	ja-dc	flowers
Viscum articulatum Burm. f.	Viscaceae	h	pe	hyp,epi	3	rv 4	ms	20	30	nv-ap	ja-ap	leafless	flowers
Scleropyrum pentandrum (Denn.) Mabb.	Santalaceae	t,l	pe	gro	2	mxf	sh,ms	25	30	fb-mr	jl-sp	ja-dc	
Acalypha brachystachya Horn.	Euphorbiaceae	h	a	gro	2	ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Acalypha indica L.	Euphorbiaceae	h	a	gro,wee	3	rv 5, da	ms	20	30	fb-sp	ap-oc	nv-oc	flowers
Alchornia tiliifolia (Bth.) M.-A.	Euphorbiaceae	l	pe	gro	3	da, sg	ms	30	30	jn-jl	oc-nv	ja-dc	
Antidesma acidum Retz.	Euphorbiaceae	l	pd	gro	3	ddf,bb/df	ms	30	30	ap-jn	jl-sp	my-dc	fruits
Antidesma ghaesembilla Gaertn.	Euphorbiaceae	t,l	pd	gro	3	bb/df	ms	30	30	my-jn	jl-ag	my-ja	fruits
Antidesma japonicum Sieb. & Zucc. var. *japonicum*	Euphorbiaceae	l,s	pd	gro	3	rv 6	ms	30	30	jl-ag		nv-ag	♂
Antidesma montanum Bl. var. *montanum*	Euphorbiaceae	l,s	pe	gro	3	bb/df,mxf	ms	30	30	ap-my	jl-ag	ja-dc	fruits
Aporosa ficifolia Baill.	Euphorbiaceae	l	pe	gro	2	mxf	ms	25	30	sp-oc	ap-my	ja-dc	
Aporosa octandra (B. -H. *ex* D. Don) Vick. var. *yunnanensis* (Pax & Hoffm.) Schot	Euphorbiaceae	t	pd	gro	3	ddf,da,sg	ms	25	30	ja-fb	ap-my	fb-nv	fruits
Aporosa villosa (Lindl.) Baill.	Euphorbiaceae	t,l	pd	gro	3	ddf	ms	30	30	ja-mr	my-jn	ap-dc	
Baliospermum solanifolium (Burm.f.) Sur.	Euphorbiaceae	s,h	pd	gro	2	da, sg	ms	30	30	jn-nv	ag-dc	jn-dc	
Blachia andamanica (Kurz) Hk. f.	Euphorbiaceae	s	pe	gro	3	mxf,da	ms	25	30	nv-dc	dc-fb	ja-dc	flowers
Blachia siamensis Gagnep.	Euphorbiaceae	s	pd	gro,rhe	3	rv 3-6	ms	20	25	jl-nv	fb-mr	nv-ag	♀,♂, fruits
Breynia vitis-ideae (Burm. f.) C.E.C. Fisch.	Euphorbiaceae	s	pd	gro	3	ddf	ms	25	30	jl-ag	oc-nv	my-nv	flowers
Bridelia harmandii Gagnep.	Euphorbiaceae	s	pd	gro	3	ddf	ms	30	30	jn-ag	ag-sp	my-nv	♂ flowers, fruits
Bridelia stipularis Bl.	Euphorbiaceae	wc,sc	pd	gro	3	bb/df,sg	ms	25	30	sp-nv	dc-fb	my-fb	
Bridelia tomentosa Bl.	Euphorbiaceae	wc	pd	gro	3	da,sg	ms	25	30	ag-nv	fb-mr	my-ja	fruits
Chaetocarpus castanocarpus (Roxb.) Thw.	Euphorbiaceae	t	pe	gro	3	mxf	ms	25	30	dc-ja	mr-ap	ja-dc	fruits

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Dalechampia falcata Gagnep.	Euphorbiaceae	v	pd	gro	3	ddf	ms	30	30	jn-ag	ag-sp	my-nv	flowers, fruits
Drypetes assamica (Hk. f.) Pax & Hoffm.	Euphorbiaceae	t	pe	gro	3	bb/df,mxf	ms	25	30	nv-dc	ja-dc	ja-dc	♀,♂
Drypetes roxburghii (Wall.) Huru.	Euphorbiaceae	t	pe	gro	3	bb/df,mxf	ms	25	30	fb-mr	oc-dc	ja-dc	♂,fruits
Drypetes thorelii Gagnep.	Euphorbiaceae	t	pe	gro	3	rv 6	ms	25	30		jl-sp	ja-ag	fruits
Euphorbia parviflora L.	Euphorbiaceae	h	a	gro	3	rv 5,ddf	ms	20	30	jl-mr	ag-ap	jn-dc	flowers,fruits
Euphorbia thymifolia L.	Euphorbiaceae	h	pe	gro,wee	2	da	ms	25	30	ja-dc	ja-dc	ja-dc	flowers,fruits
Fluggea virosa (Roxb. *ex* Willd.) Voigt	Euphorbiaceae	l,s	pd	gro	3	rv 5-6, bb/df, da, sg	ms	25	30	mr-ag	jn-sp	my-fb	♀,♂, fruits
Homonoia riparia Lour.	Euphorbiaceae	s	pd	gro,rhe	5	rv 2-5	ms	20	25	mr-ap	jl-dc	ja-jn	♀,♂, fruits
Hymenocardia punctata Wall. *ex* Lindl.	Euphorbiaceae	l,s	pd	gro	3	bb/df,mxf,sg	ms	25	30	mr-my	ag-sp	my-fb	♂,♀
Mallotus cuneatus Ridl.	Euphorbiaceae	l	pe	gro	3	bb/df,mxf	ms,ry	25	30	mr-my	jl-nv	ja-dc	♂,fruits
Mallotus nudiflorus (L.) Kul. & Welz. (*Trewia nudiflora* L.)	Euphorbiaceae	t	pd	gro	3	rv 6	ms	25	30	fb-ap	sp-oc	my-nv	
Mallotus philippensis (Lmk.) M.-A.	Euphorbiaceae	t	pd	gro	2	bb/df	ms	30	30	nv-dc	ja-mr	my-mr	
Mallotus repandus (Willd.) M.-A.	Euphorbiaceae	wc	pe	gro	3	da,sg,bb/df	ms	25	30	ja-fb	ap-my	ja-dc	
Pantadenia adenanthera Gagnep.	Euphorbiaceae	s	pd	gro	3	bb/df,da	ms	25	30	jl-ja	nv-mr	my-ap	♂,fruits
Phyllanthus amarus Schum. & Thonn.	Euphorbiaceae	h	a	gro,wee	3	da	sh,ms	25	30	ja-dc	ja-dc	ja-dc	flowers,fruits
Phyllanthus emblica L.	Euphorbiaceae	t	pd	gro	3	ddf,bb/df	sh,ms	25	30	fb-mr	sp-dc	mr-dc	
Phyllanthus jullienii Beille	Euphorbiaceae	s	pd	gro,rhe	4	rv 2-4	ms	20	25	nv-dc	mr-my	oc-ap	flowers
Phyllanthus pulcher Wall. *ex* M.-A.	Euphorbiaceae	l	pd	gro	2	bb/df,mxf	ms	25	30	sp-nv	nv-dc	nv-ja	♂,fruits
Phyllanthus reticulatus Poit.	Euphorbiaceae	sc,wc	pd	gro	3	rv 5,da,sg	ms	20	30	jl-ag	sp-nv	my-dc	
Phyllanthus urinaria L.													Euph
Riccinus communis L.	Euphorbiaceae	h	a	gro,int,nat	3	da	ms	25	30	jn-sp	jl-oc	my-dc	
Sauropus androgynus (L.) Merr.	Euphorbiaceae	l	pd	gro	2	bb/df,da	ms	25	30	ag-sp	oc-dc	my-dc	fruits
Suregada multiflora (A. Juss.) Baill. var. *multiflora*	Euphorbiaceae	t	pe	gro	2	mxf	ms	25	30	mr-my	ap-jn	ja-dc	♂,♀
Thyrsanthera suborbicularis Pierre *ex* Gagnep.	Euphorbiaceae	h	pd	gro	3	ddf	ms	25	30	mr-ap	ap-jn	my-dc	♂,♀
Trema orientalis (L.) Bl.	Ulmaceae	t	pe	gro	3	da,sg	sh,ms	25	30	mr-ap	my-jl	ja-dc	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Artocarpus ?lakoocha Roxb.	Moraceae	t	pe	gro	2	bb/df, mxf	ms	30	30			ja-dc	
Ficus alongensis Gagnep.	Moraceae	t	pe	gro,epi,str	3	rv 6, mxf	ms	20	30	ap-ag	my-sp	ja-dc	figs
Ficus benjamina L.	Moraceae	t	pe	gro,epi,str	3	rv 4, 6, mxf	ms	25	30	fb-mr	mr-ap	ja-dc	figs
Ficus fistulosa Reinw. *ex* Bl.	Moraceae	t	pd	gro	3	da,sg	sh,ms	25	30	ja-dc	ja-dc	my-fb	
Ficus heterophylla L. f.	Moraceae	cr,wc	pe	gro	3	rv 5-6,mxf,da	ms	25	30	ja-dc	ja-dc	ja-dc	figs
Ficus hispida L. f.	Moraceae	l,t	pe	gro	3	da,sg	ms	25	30	ja-dc	ja-dc	ja-dc	figs
Ficus kurzii King	Moraceae	t	pe	gro,epi,str	3	rv 6, mxf	ms	25	30	ja-ap	ja-ap	ja-dc	figs
Ficus racemosa L.	Moraceae	t	pd	gro	3	da,sg	ms	25	30	ja-dc	ja-dc	oc-ag	figs
Ficus rumphii Bl.	Moraceae	t	pd	gro,epi,str	3	rv 4, 6	ms	20	25	mr-ap(ag)	ap-my	mr-dc	figs
Ficus subpisocarpa Gagnep.	Moraceae	t	pd	gro,epi,str	3	ddf	ms	25	30	fb-mr	mr-ap	my-nv	figs
Ficus virens Ait	Moraceae	t	pe	epi,str	3	rv 4,6,streams	sh,ms	25	30	sp-mr	sp-ap	ja-dc	figs
Strepblus asper Lour. var. *asper*	Moraceae	l,t	pe	gro	3	rv 6, mxf	ms	25	30	ja-mr	de-mr	ja-dc	flowers,fruits
Laportea interrupta (L.) Chew	Urticaceae	h	a	gro,wee	3	bb/df,da	ms	25	30	jn-nv	ag-dc	my-dc	fruits
Pouzoulzia zeylanica (L.) Benn.	Urticaceae	h	a	gro	2	rv 4-6	ms	25	30	jl-ag	sp-oc	ja-ag	flowers
Salix tetrasperma Roxb.	Salicaceae	t	pd	gro,rhe	2	rv 6	ms	20	25	nv-dc	dc-ja	nv-ag	
Angiospermae, Monocotyledoneae													
Hydrilla verticillata (L. f.) Roy.	Hydrocharitaceae	h	a	aqu	2	ponds in ddf	ms	30	30			jn-nv	
Lagarosiphon roxburghii Bth.	Hydrocharitaceae	h	a	aqu,gro	2	ponds in bb/df	ms	20	25	fb-mr	mr-ap	jl-ap	flowers,fruits
Ottellia lanceolata (Gagnep.) Dandy	Hydrocharitaceae	h	a	aqu,gro	2	ponds in bb/df	ms	25	30	ag-oc	dc-nv	my-dc	flowers,fruits
Vallisneria gigantea Greab.	Hydrocharitaceae	h	a	aqu	3	rv 1	ms	20	20	mr-my	ap-jn	nv-jn	
Sagittaria guayanensis Humb. ssp. *lappula* (D. Don) Bogin	Alismataceae	h	a	aqu,gro	3	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Sagittaria trifolia L.	Alismataceae	h	a	aqu,gro	2	ponds in bb/df, rv 5	ms	20	25	fb-mr	mr-ap	ja-dc	flowers,fruits
Potamogeton crispus L.	Potamogetonaceae	h	a	aqu	3	rv 1	ms	20	20	dc-ja	mr	ja-dc	fruits
Najas indica (Willd.) Cham.	Najadaceae	h	pe	aqu	3	rv 1	ms	20	20	ja-fb	mr-ap	ja-dc	fruits
Belosynapsis ciliata (Bl.) R. Rao	Commelinaceae	h	a	gro	2	bb/df,da	ms	25	30	ag-nv	nv-ja	my-ja	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Commelina diffusa Burm. f.	Commelinaceae	h	a	gro	3	bb/df,da	ms	25	30	jn-ag	ag-oc	ja-dc	flowers
Cyanotis axillaris (L.) D. Don	Commelinaceae	h	a	gro	2	bb/df,da	ms	25	30	jl-oc	ag-nv	my-dc	flowers
**Murdannia discreta* (Craib) Thit. & Faden	Commelinaceae	h	pd	gro	3	ddf	ms	30	30	jl-ag	ag-sp	my-dc	fruits
Murdannia edulis (Stokes) Faden	Commelinaceae	h	pd	gro	3	ddf, bb/df	ms	30	30	jl-ag	ag-sp	my-nv	flowers, fruits
Murdannia gigantea (Vahl) Bruck.	Commelinaceae	h	pd	gro	3	ddf	ms	30	30	jn-ag	jl-sp	my-nv	flowers, fruits
Murdannia nudiflora (L.) Bren.	Commelinaceae	h	pd	gro	3	ddf	ms	30	30	jn-ag	ag-sp	my-nv	flowers, fruits
Eriocaulon sexangulare L.	Eriocaulaceae	h	a	gro	3	ddf,bb/df	ms	25	30	sp-nv	oc-dc	my-dc	flowers
Eriocaulon sieboldianum Sieb. & Zucc. *ex* Steud.	Eriocaulaceae	h	a	gro	3	ddf,bb/df	ms	25	30	sp-nv	oc-dc	my-dc	flowers
Alpinia malaccensis (Burm. f.) Rosc.	Zingiberaceae	h	pe	gro	2	da, sg	ms	30	30	mr-my	sp-oc	ja-dc	
Costus speciosus (Koen.) J. E. Sm.	Zingiberaceae	h	pd	gro	3	ddf,bb/df	ms	25	30	jl-sp	oc-nv	my-dc	flowers, fruits
Curcuma aurantiaca van Zijp	Zingiberaceae	h	pd	gro	3	bb/df	ms	25	30	jl-ag		my-nv	flowers
Curcuma gracillima Gagnep.	Zingiberaceae	h	pd	gro	3	ddf	ms	30	30	jl-ag		my-nv	flowers
Curcuma zedoaria (Christm.) Rosc.	Zingiberaceae	h	pd	gro	3	ddf	ms	30	30	ap-my	jl-ag	my-nv	
Curcuma (07-431)	Zingiberaceae	h	pd	gro	3	ddf,bb/df	ms	30	30	jl-ag	sp-oc	my-nv	flowers
Curcuma (07-443)	Zingiberaceae	h	pd	gro	2	bb/df	ms	30	30	jl-ag		my-nv	flowers
Globba schomburgkii Hk. f. var. *schomburgkii*	Zingiberaceae	h	pd	gro	3	ddf,bb/df	ms	25	30	jl-dc	oc-nv	my-dc	flowers
Kaempferia angustifolia Rosc.	Zingiberaceae	h	pd	gro	3	ddf,bb/df	ms	30	30	jl-ag	oc-nv	my-nv	flowers
Kaempferia siamensis Siri.	Zingiberaceae	h	pd	gro	3	ddf	ms	30	30	jl-ag	sp-oc	my-nv	flowers
Stahlianthus thorelii Gagnep.	Zingiberaceae	h	pd	gro	2	ddf	ms	30	30	ap-my	jl-ag	my-nv	fruits
Zingiber montanum (Koen.) Link *ex* Dietr.	Zingiberaceae	h	pd	gro,cul,int	2	bb/df	ms	30	30	ag-sp		my-dc	
Zingiber pellitum Gagnep.	Zingiberaceae	h	pd	gro	2	bb/df	ms	30	30	jl-ag		my-nv	flowers
Zingiber zerumbet (L.) Sm. var. *zerumbet*	Zingiberaceae	h	pd	gro	3	ddf,bb/df	ms	30	30	jl-ag	sp-oc	my-nv	flowers
Halopegia brachystachys Craib	Marantaceae	h	pd	gro	4	ddf,bb/df	ms	30	30	jl-ag	sp-oc	my-nv	flowers
Chloropytum intermedium Craib var. *intermedium*	Liliaceae	h	pd	gro	3	ddf	ms	30	30	jl-sp	oc-nv	my-nv	
Gloriosa superba L.	Liliaceae	v	pd	gro,int,nat	2	bb/df	ms	30	30	jl-ag		my-dc	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Liriope spicata Lour.	Liliaceae	h	pd	gro	3	bb/df	ms	30	30	jl-ag	ag-sp	my-nv	flowers, fruits
Hypoxis aurea Lour.	Amaryllidaceae	h	pd,ped	gro	2	ddf,bb/df	ms	30	30	jn-ag	jl-sp	my-nv	flowers
Monochoria vaginalis (Burm. f.) Presl	Pontederiaceae	h	a	aqu,gro	3	ponds,wet areas	ms	20	25	ja-ag	mr-sp	nv-oc	flowers,fruits
Smilax cambodiana Gagnep.	Smilacaceae	v	pe	gro	3	da	ms	30	30		jl-ag	ja-dc	fruits
Smilax verticalis Gagnep.	Smilacaceae	v	pd	gro	3	ddf	ms	30	30	ap-my	jl-ag	my-nv	fruits
Alocasia odora C. Koch	Araceae	h	pd	gro	2	bb/df	ms	25	30	ag-sp	nv-dc	my-dc	fruits
Amorphophallus coudercii (Bogn.) Bogn.	Araceae	h	pd	gro	1	bb/df	ms	25	30	mr		my-nv	flowers
Amorphophallus harmandii Engl. & Gehrm.	Araceae	h	pd	gro	3	bb/df	ms	30	30	jl		jn-oc	leaves
Amorphophallus hemicryptus Hett.	Araceae	h	pd	gro	2	bb/df	sh,ms	25	30	nv		jn-oc	flowers, leaves
**Amorphophallus koratensis* Gagnep.	Araceae	h	pd	gro	2	bb/df	ms	25	30	mr-ap		my-oc	flowers, leaves
**Cryptocoryne crispatula* Engl. var. *crispatula*	Araceae	h	pd	gro,rhe, rarely epl	4	rv 2-3	ms	20	25	nv		sp-ap	flowers
Pothos scandens L.	Araceae	v,cr	pe	gro	2	mxf	ms	25	30	oc-dc	ja-fb	ja-dc	flowers
Rhaphidophora peepla (Roxb.) Schott	Araceae	v,cr	pe	epi	3	bb/df	ms	25	30	jl-sp	oc-mr	ja-dc	fruits
Typhonium flagelliforme (Lodd.) Bl.	Araceae	h	pd	aqu, gro	2	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers
**Typhonium laoticum* Gagnep.	Araceae	h	pd	gro	2	bb/df	ms	30	30	jl-ag	ag-sp	my-nv	flowers, fruits
Lemna aequinoctalis Welw.	Lemnaceae	h	a	aqu	3	ponds	ms	25	30			sp-mr	
Stemona tuberosa Lour. var. *tuberosa*	Stemonaceae	v	pd	gro	3	ddf, bb/df	ms	25	30	ap-jl	jn-sp	my-dc	flowers
Dioscorea alata L.	Dioscoreaceae	v	pd	gro	3	da,sg	ms	25	30	sp-nv	nv-fb	my-dc	flowers,fruits
Dioscorea glabra L. var. *glabra*	Dioscoreaceae	v	pd	gro	3	bb/df,da,sg	ms	25	30	sp-dc	nv-dc	my-dc	fruits
Dioscorea hispida Denn. var. *hispida*	Dioscoreaceae	v	pd	gro	3	bb/df	ms	25	30	mr-ap	oc-nv	my-dc	fruits
Calamus rudentum Lour.	Palmae	wc	pe	gro	3	bb/df,mxf	ms	25	30	sp-oc	mr-ap	ja-dc	fruits
Calamus siamensis Becc. var. *siamensis*	Palmae	wc	pe	gro	4	mxf	sh,ms	25	30			ja-dc	
Calamus viminalis Willd.	Palmae	wc	pe	gro	2	bb/df,mxf	ms	25	30	sp-oc	nv-dc	ja-dc	
Caryota maxima Bl.	Palmae	t	pe	gro	1	da	ms	30	30	ja-dc	ja-dc	ja-dc	
Caryota mitis Lour.	Palmae	t	pe	gro	2	mxf	ms	25	30	ja-dc	ja-dc	ja-dc	

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Licuala spinosa Thunb.	Palmae	l	pe	gro	2	mxf	ms	25	30	ja-fb	my-jn	ja-dc	imm. fruits
Burmannia coelestis D. Don	Burmanniaceae	h	a	gro	3	wet areas in ddf	ms	25	30	sp-nv	nv-dc	jl-dc	flowers
Burmannia wallichii (Miers) Hk. f.	Burmanniaceae	h	a	gro,sap	2	bb/df	ms	25	30	oc-nv	nv-dc	leafless	flowers
Apostasia wallichii R. Br.	Orchidaceae	h	pe	gro	2	bb/df	ms	25	30	jl-ag	nv-dc	ja-dc	fruits
**Brachycorythis helferi* (Rchb. f.) Summ.	Orchidaceae	h	pd	gro	3	ddf,bb/df	ms	30	30	jl-ag		my-nv	flowers
Brachycorythis laotica (Gagnep.) Summ.	Orchidaceae	h	pd	gro	3	ddf,bb/df	ms	30	30	jl-ag		my-nv	flowers
Bulbophyllum	Orchidaceae	h	pe	epi	3	mxf	ms	25	30			ja-dc	
Cleisomeria pilosulum (Gagnep.) Seid. & Garay	Orchidaceae	h	pe	epi	3	ddf	ms	30	30	jl-ag		ja-dc	flowers
Dendrobium venustum Teijs. & Binn.	Orchidaceae	h	pd	epi	2	bb/df	ms	25	30	oc-dc	nv-fb	my-fb	flowers
Habenaria dentata (Sw.) Schltr.	Orchidaceae	h	pd	gro	2	mxf,da	ms	25	30	oc-dc	jn-dc	my-dc	flowers
Habenaria khasiana Hk. f.	Orchidaceae	h	pd	gro	3	ddf	ms	30	30	jn-ag		my-nv	flowers
Habenaria lucida Wall. ex Lindl.	Orchidaceae	h	pd	gro	3	ddf,bb/df	ms	30	30	jl-ag		my-nv	flowers
Habenaria mandersii Coll. & Hemsl.	Orchidaceae	h	pd	gro	3	ddf	ms	30	30	jl-ag		my-nv	flowers
Habenaria rostellifera Rchb. f.	Orchidaceae	h	pd	gro	3	ddf	ms	30	30	jl-ag		my-nv	flowers
Habenaria rumphii (Brogn.) Lindl.	Orchidaceae	h	pd	gro	3	ddf	ms	30	30	jl-ag		my-nv	flowers
**Habenaria viridiflora* (Rottl. *ex* Sw.) R. Br.	Orchidaceae	h	pd	gro	3	ddf	ms	30	30	jl-ag		my-nv	flowers
Liparis campylostalix Rchb. f.	Orchidaceae	h	pd	gro	2	bb/df	ms	25	30	ag-oc	nv-fb	my-dc	fruits
**Liparis rheedii* (Bl.) Lindl.	Orchidaceae	h	pd	gro	3	bb/df	ms	30	30	jl-ag		my-nv	flowers
**Liparis siamensis* Rol. *ex* Dow.	Orchidaceae	h	pd	gro	3	mxf	ms	30	30	jl-ag		my-nv	flowers
Luisia thailandica Seid.	Orchidaceae	h	pe	epi	2	bb/df	ms	25	30	mr-ap	my-jl	ja-dc	flowers
Nervilia aragoana Gaud.	Orchidaceae	h	pd	gro	2	ddf, bb/df	ms	25	30	ar-my		my-dc	
**Nervilia calcicola* Kerr	Orchidaceae	h	pd	gro	2	bb/df	ms	30	30			my-nv	
Nervilia punctata (Bl.) Schltr.	Orchidaceae	h	pd	gro	2	bb/df	ms	30	30	ap-my		my-nv	leaves
Peristylus constrictus (Lindl.) Lindl.	Orchidaceae	h	pd	gro	2	bb/df	ms	25	30	jl-ag	oc-nv	my-nv	flowers
**Vandopsis gigantea* (Lindl.) Pfitz.	Orchidaceae	h	pe	epi	2	mxf	ms	25	30	mr-ap		ja-dc	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Carex indica L. var. *indica*	Cyperaceae	h	pd	gro	3	mxf	ms	25	30	mr-ap	jn-sp	mr-dc	flowers
Carex tricephala Boeck.	Cyperaceae	h	pd	gro	3	ddf,bb/df	ms	30	30	jl-ag	ag-sp	my-nv	flowers, fruits
Cyperus brevifolius (Rottb.) Hassk.	Cyperaceae	h	pd	gro	3	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Cyperus castaneus Willd.	Cyperaceae	h	a	gro	3	wet areas in ddf	ms	30	30	jn-ag	jl-sp	my-nv	flowers, fruits
Cyperus compactus Retz.	Cyperaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jn-sp	jl-oc	my-nv	flowers, fruits
Cyperus cuspidatus Kunth.	Cyperaceae	h	a	gro	3	rv 5	ms	20	25	jn-ag	jl-sp	ja-ag	flowers, fruits
Cyperus iria L.	Cyperaceae	h	a	aqu, gro, wee	3	rv 5, ponds in ddf, da	ms	25	30	jl-oc	ag-nv	jn-nv	
Cyperus kyllingia Endl.	Cyperaceae	h	pe	gro, wee	3	da, sg	ms	25	30	my-dc	jn-ja	ja-dc	
Cyperus laxus Lmk. var. *laxus*	Cyperaceae	h	pe	gro	3	bb/df,da	ms	25	30	jl-dc	sp-dc	ja-dc	flowers
Cyperus leucocephalus Retz.	Cyperaceae	h	pd	gro	3	ddf	ms	30	30	jl-sp	ag-oc	my-dc	flowers, fruits
Cyperus pilosus Vahl	Cyperaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jn-sp	jl-oc	my-nv	flowers, fruits
Cyperus pygmaeus Rottb.	Cyperaceae	h	a	gro	2	ddf,da	ms	25	30	oc-dc	nv-dc	ja-dc	flowers
Cyperus tenuispica Steud.	Cyperaceae	h	a	gro	3	bb/df	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Cyperus triceps (Rottb.) Engl.	Cyperaceae	h	pd	gro	3	wet areas in ddf	ms	30	30	jn-ag	jl-sp	my-nv	flowers, fruits
Diplacrum caricinum R. Br.	Cyperaceae	h	a	gro	3	wet areas in ddf	ms	30	30	jn-sp	jn-sp	my-nv	flowers, fruits
Eleocharis acutangula (Roxb.) Schult.	Cyperaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Eleocharis dulcis (Burm. f.) Hensch. var. *dulcis*	Cyperaceae	h	a	aqu, gro	2	ponds in ddf	ms	30	30			my-nv	
Fimbristylis adenolepis Kern	Cyperaceae	h	a	gro	3	ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Fimbristylis aestivalis (Retz.) Vahl var. *aestivalis*	Cyperaceae	h	a	gro	3	rv 5	ms	20	25	ja-my	fb-jn	ja-jn	flowers
Fimbristylis bisumbellata (Forssk.) Bub.	Cyperaceae	h	a	gro	3	bb/df	ms	30	30	jn-ag	jl-sp	my-nv	flowers, fruits
**Fimbristylis brunneoides* Kern	Cyperaceae	h	a	gro	3	rv 4-5	ms	20	30	ja-ap	fb-my	nv-jn	flowers
Fimbristylis cymosa R. Br.	Cyperaceae	h	pd	gro,rhe	3	rv 2-3	ms	20	25	fb-ap	oc-nv	nv-jn	flowers,fruits
Fimbristylis dichotoma (L.) Vahl ssp. *dichotoma*	Cyperaceae	h	pd	gro	3	ddf,bb/df	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Fimbristylis dipascea (Rottb.) Cl.	Cyperaceae	h	a	gro	3	rv 5	ms	20	25	ja-my	fb-jn	ja-jn	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Fimbristylis gracilenta Hance	Cyperaceae	h	a	gro	3	bb/df	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
**Fimbristylis jucunda* (Cl.) Kern	Cyperaceae	h	a	gro	3	rv 2-4	ms	20	25	ja-mr	fb-ap	nv-jn	flowers
Fimbristylis miliacea (L.) Vahl	Cyperaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Fimbristylis schoenoides (Retz.) Vahl	Cyperaceae	h	pd	gro	3	wet areas in ddf	ms	30	30	jn-sp	jl-oc	my-nv	flowers, fruits
Fimbristylis tetragona R. Br.	Cyperaceae	h	a	aqu, gro	3	ponds in ddf	ms	30	30	jn-sp	jl-oc	my-nv	flowers, fruits
Liphocarpa hemisphaerica (Roth) Goet.	Cyperaceae	h	a	gro	3	wet areas in ddf	ms	30	30	jn-sp	jl-oc	my-nv	flowers, fruits
Liphocarpa microcephala (R. Br.) Kunth	Cyperaceae	h	a	gro	3	wet areas in ddf	ms	30	30	jn-ag	jl-sp	my-nv	flowers, fruits
Rhynchospora longisetis R. Br.	Cyperaceae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers,fruits
Rhynchospora rubra (Lour.) Mak.	Cyperaceae	h	pd	gro	3	wet areas in ddf	ms	30	30	jn-sp	jl-oc	my-dc	flowers, fruits
Scleria levis Retz.	Cyperaceae	h	pd	gro	3	ddf, bb/df, mxf, da	ms	25	30	jn-oc	jl-nv	my-nv	flowers, fruits
Scleria lithosperma (L.) Sw. var. *lithosperma*	Cyperaceae	h	pe	gro	2	bb/df	ms	25	30	sp-nv	nv-ja	ja-dc	fruits
Scleria neesii Kunth	Cyperaceae	h	a	gro	3	wet areas in ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Scleria psilorrhiza Cl.	Cyperaceae	h	pd	gro		wet areas in ddf	ms	30	30	jn-sp	ag-oc	my-nv	flowers, fruits
Alloteropsis cimicina (L.) Stapf	Gramineae	h	a	gro	3	ddf, da	ms	30	30	jn-ag	jl-sp	my-nv	flowers, fruits
Andropogon chinensis (Nees) Merr.	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Apocopsis cochinchinensis A. Camus	Gramineae	h	a	gro	3	wet areas in ddf	ms	30	30	jn-sp	jl-oc	my-nv	flowers
Aristida chinensis Munro	Gramineae	h	pd	gro	3	ddf	ms	25	30	oc-nv	dc-ja	my-dc	flowers
Aristida setacea Retz.	Gramineae	h	pd	gro	3	bb/df	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Arundinella setosa Trin. var. *setosa*	Gramineae	h	pd	gro	3	ddf	ms	30	30	jn-ag	ag-sp	my-nv	flowers, fruits
Capillipedium annamense A. Camus	Gramineae	h	pd	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Capillipedium assimile (Steud.) A. Camus	Gramineae	h	pd	gro	3	ddf	ms	25	30	sp-nv	nv-dc	my-dc	flowers
Capillipedium cinctum (Steud.) A. Camus	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Chrysopogon nemoralis (Balan.) Holtt.	Gramineae	h	pd	gro	4	ddf, bb/df	ms	25	30	jl-sp	sp-oc	jn-fb	flowers
Cyrtococcum accrescens (Trin.) Stapf	Gramineae	h	a	gro	3	bb/df,da	ms	25	30	sp-nv	nv-dc	my-dc	flowers
Dactyloctenium aegyptium (L.) P. Beauv.	Gramineae	h	a	gro, wee	3	rv 5, da	ms	25	30	jn-oc	jl-nv	my-nv	flowers
Dichanthium caricosum (L.) A. Camus	Gramineae	h	a	gro	3	rv 5	ms	20	25	ja-fb	fb-mr	nv-jn	flowers
Digitaria bicornis (Lmk.) Roem. & Schult.	Gramineae	h	a	gro	3	rv 5	ms	20	25	ja-ag	ja-ag	ja-ag	flowers
Digitaria radicosa (Presl) Miq.	Gramineae	h	a	gro	3	rv 5, ddf, da	ms	20	30	ja-ag	fb-sp	nv-ag	flowers
Digitaria violascens Link	Gramineae	h	a	gro	2	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Echinochloa colona (L.) Link	Gramineae	h	a	aqu, gro	3	rv 5, ponds in ddf	ms	25	30	jn-ag	jl-sp	ja-sp	flowers, fruits
Eleusine indica (L.) Gaertn.	Gramineae	h	a	gro,wee	3	rv 5, da	ms	20	30	nv-ap	dc-my	nv-jn	
Enteropogon dolichostachya (Lag.) Keng *ex* Laza.	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Eragrostis bipinnata (L.) Musc.	Gramineae	h	a	gro	3	wet areas in ddf	ms	30	30	jn-sp	jl-oc	my-nv	flowers, fruits
Eragrostis pilosa (L.) P. Beauv.	Gramineae	h	a	gro	3	ddf, bb/df,da	ms	25	30	jl-nv	ag-nv	my-dc	flowers
Eragrostis unioloides (Retz.) Nees *ex* Steud.	Gramineae	h	a	gro	3	ddf	ms	25	30	sp-nv	nv-dc	my-dc	flowers
Eremochloa ciliaris (L.) Merr.	Gramineae	h	pd	gro	3	ddf	ms	30	30	jl-sp	ag-oc	my-nv	flowers
Eulalia velutina (Munro) O.K.	Gramineae	h	pd	gro	3	ddf	ms	25	30	sp-nv	nv-dc	my-dc	flowers
Eulaliopsis binata (Retz.) C. E. Hubb.	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Gymnopogon delicatulus (Cl.) Bor	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Hemisorghum mekongense (A. Camus) C.E. Hubb. *ex* Bor	Gramineae	h	a	gro	3	rv 5	ms	25	30	jl-ag	ag-sp	ja-ag	flowers
Heteropogon contortus (L.) P. Beauv. *ex* Roem. & Schult.	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Hyparrhena hirta (L.) Stapf	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	dc	my-dc	flowers
Imperata cylindrica (L.) P. Beauv. var. *major* (Nees) C. E. Hubb. *ex* Hubb. & Vaugh.	Gramineae	h	pd	gro	3	da, sg	ms	30	30	jl-oc	ag-nv	my-dc	
Ischaemum indicum (Houtt.) Merr.	Gramineae	h	pd	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Leptochloa chinensis (L.) Nees	Gramineae	h	a	gro	3	rv 5, bb/df	ms	20	30	ja-dc	ja-dc	ja-dc	flowers
Lophaterum gracile Brongn. var. *gracile*	Gramineae	h	a	gro	3	bb/df,mxf	ms	25	30	sp-nv	nv-dc	my-dc	flowers
Microchloa indica (L. f.) P. Beauv.	Gramineae	h	a	gro	3	ddf	ms	30	30	jl-ag	ag-sp	my-nv	flowers
Mnestithea laevis (Retz.) Kunth var. *cochinchinensis* (Lour.) Kon. & Sos.	Gramineae	h	pd	gro	3	ddf	ms	30	30	jn-ag	jl-sp	my-nv	flowers, fruits
Mnesithea striata (Nees *ex* Steud.) Kon. & Sos.	Gramineae	h	pd	gro	3	bb/df,da	ms	25	30	jl-nv	ag-dc	my-dc	flowers
Oplismenus compositus (L.) P. Beauv.	Gramineae	h	a	gro	3	bb/df,da	ms	25	30	sp-nv	oc-dc	my-dc	flowers
Oryza sativa L.	Gramineae	h	a	aqu, gro	3	wet areas in ddf	ms	30	30	jl-ag	ag-sp	my-nv	flowers, fruits
Panicum luzonense Presl	Gramineae	h	a	gro	3	bb/df	ms	30	30	jl-sp	ag-oc	my-nv	flowers, fruits
Panicum notatum Retz.	Gramineae	h	pd	gro	3	bb/df,da	ms	25	30	sp-nv	oc-dc	my-dc	flowers
Panicum trachyrhachis Bth.	Gramineae	h	a	gro	3	ddf	ms	25	30	sp-nv	nv-dc	mydc	flowers
Paspalum scrobiculatum L.	Gramineae	h	a	gro	3	bb/df,da	ms	25	30	jl-nv	ag-dc	my-dc	flowers
Phragmites vallatoria (Pluk. *ex* L.) Veldk.	Gramineae	h	pe	gro,wee	3	da,sg	sh,ms	25	30	nv-fb	dc-mr	ja-dc	
Polytoca digitata (L. f.) Druce	Gramineae	h	pd	gro	3	ddf	ms	25	30	oc-nv	dc	my-dc	flowers
Rottboellia exalata L. f.	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Saccharum arundinaceum Retz.	Gramineae	h	pe	gro	4	streams, wet areas, rv 5, da	ms	25	30	sp-nv	nv-dc	ja-dc	flowers
Saccharum spontaneum L.	Gramineae	h	pd	gro	3	streams, wet areas, rv 5, da	ms	20	30	dc-mr	fb-ap	nv-jn	flowers
Sacciolepis indica (L.) A. Chase	Gramineae	h	a	gro	3	ddf	ms	30	30	jn-sp	jl-oc	my-dc	flowers, fruits
Schizachyrium brevifolium (Sw.) Nees	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Sclerostachya fusca (Roxb.) A. Camus	Gramineae	h	pd	gro	4	wet areas,sg	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Setaria parviflora (Poir.) Kerg.	Gramineae	h	a	gro	4	ddf, bb/df	ms	25	30	jl-nv	ag-dc	my-dc	flowers
Sorghum propinquum (Kunth) Hitch.	Gramineae	h	a	gro	3	wet areas,sg	ms	25	30	oc-nv	nv-dc	my-dc	flowers
Themeda arundinacea (Roxb.) Ridl.	Gramineae	h	a	gro	3	ddf	ms	25	30	oc-nv	nv-dc	my-dc	flowers

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Thysanolaena latifolia (Roxb. *ex* Horn.) Honda	Gramineae	h	pe	gro,wee	4	da,sg	ms	25	30	ag-oc	sp-nv	ja-dc	
Bambusa bambos (L.) Voss. *ex* Vilm.	Gramineae, Bambusoideae	h	pe	gro	5	bb/df,da	ms	25	30	fb-mr	mr-ap	ja-dc	
Dendrocalamus sp.	Gramineae, Bambusoideae	h	pe	gro	2	bb/df	ms	25	30			my-dc	
Thyrsostachys siamensis (Kurz *ex* Munro) Gamb.	Gramineae, Bambusoideae	h	pd	gro	3	bb/df	ms	30	30	mr-ap		my-dc	
Vietnamosasa ciliata (A. Camus) Nguyen	Gramineae, Bambusoideae	h	pd	gro	4	ddf	ms	25	30	sp-oc		my-dc	
Pteridophyta													
Selaginella roxburghii (Hk. & Grev.) Spring var. *roxburghii*	Selaginellaceae	h	a	gro	2	bb/df, mxf,sg	ms	25	30	ag-nv	ag-nv	my-dc	sporangia
Helminthostachys zeylanica (L.) Hk.	Ophioglossaceae	h	pd	gro	2	bb/df	ms	30	30	jl-ag	jl-ag	my-nv	sori
Ophioglossum gramineum Willd. var. *gramineum*	Ophioglossaceae	h	pd	gro	2	ddf	ms	30	30	jl-sp	jl-sp	jn-sp	
Ophioglossum petiolatum Hk.	Ophioglossaceae	h	pd	gro	2	bb/df,da	ms	25	30	jl-dc	jl-nv	jn-dc	sori
Lygodium flexuosum (L.) Sw.	Schizaeaceae	v	pd	gro	3	ddf, bb/df	ms	25	30	jl-nv	jl-nv	my-dc	sori
Adiantum philippense L.	Parkeriaceae	h	pd	gro	3	bb/df,ddf	ms	25	30	ag-nv	ag-nv	my-dc	sori
Adiantum zollingeri Mett. *ex* Kuhn	Parkeriaceae	h	pd	gro	3	bb/df,ddf	sh,ms	25	30	sp-dc	sp-dc	my-dc	sori
Ceratopteris thalictroides (L.) Brongn.	Parkeriaceae	h	pd	aqu,gro	3	ponds in bb/df	ms	25	30	sp-nv	sp-nv	jn-dc	sori
Cheilanthes belangeri (Bory) C. Chr.	Parkeriaceae	h	pd	gro	3	bb/df	ms	25	30	ag-nv	ag-nv	my-dc	sori
Hemionitis arifolia (Burm. f.) Moore	Parkeriaceae	h	pd	gro	2	bb/df,mxf	ms	25	30	sp-nv	sp-nv	my-dc	sori
Pteris heteromorpha Fee	Pteridaceae	h	pe	gro	3	bb/df	ms	25	30	sp-nv	sp-nv	ja-dc	sori
Diplazium esculentum (Retz.) Sw.	Athyriaceae	h	pd	gro	4	rv 2-3	ms	20	25	ap-my	ap-my	nv-jn	sori
Drynaria bonii Christ	Polypodiaceae	h	pd	gro,epi,epl	2	rocks in bb/df	ms	25	30	sp-nv	sp-nv	my-dc	sori
Drynaria quercifolia (L.) J. Sm.	Polypodiaceae	h	pd	epi	3	rv 6,bb/df,mxf	ms	25	30	ag-oc	ag-oc	my-ja	
Platycerium wallichii Hk.	Polypodiaceae	h	pe	epi	1	mxf	ms	30	30	oc-ap	oc-ap	ja-dc	
Pyrrosia lanceolata (L.) Farw.	Polypodiaceae	h,cr	pe	epi	3	bb/df, mxf	sh,ms	25	30	jl-dc	jl-dc	ja-dc	sori
Pyrrosia stigmosa (Sw.) Ching	Polypodiaceae	h	pe	epi	3	rv 6, mxf	ms	25	30	mr-nv	mr-nv	ja-dc	sori

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Species	Family	Habit	Aped	Life-mode	Abundance	Habitat	Bedrock	Lower Elevation (m)	Upper Elevation (m)	Flowering Month	Fruiting Month	Leafing Month	Collected
Bryophyta													
Bryum coronatum Schwaegr.	Bryaceae	h	pe	epi	3	bb/df	ms	25	30	ag-nv	ag-nv	ja-dc	capsules
Fisssidens zollingeri Mont.	Fissidentaceae	h	pe	gro	2	wet areas in bb/df	ms	25	30	sp-nv	sp-nv	ja-dc	capsules
Ochrobryum sp.	Leucobryaceae	h	pe	epi	3	bb/df	ms	25	30	nv-mr	nv-mr	ja-dc	capsules
Octoblepharum albidum Hedw.	Octoblepharaceae	h	pe	epi	3	bb/df	ms	30	30	jn-sp	jn-sp	ja-dc	capsules
Macromitrum zollingeri Mitt. *ex* Dozy & Molk.	Orthotrichaceae	h	pe	epi	3	streams in bb/df,mxf	ms	25	30	sp-nv	sp-nv	ja-dc	capsules
Riccia sp.	Ricciaceae	h	a	gro	2	rv 6,streams, wet areas	ms	20	25	nv-dc	jn-mr	nv-jn	capsules
Taxithelium nepalense (Schwaegr.) Broth.	Sematophyllaceae	h	pe	epi	3	bb/df	ms	25	30	ag-nv	ag-nv	ja-dc	capsules

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Full Paper

Antimicrobial property and antioxidant composition of crude extracts of *Pueraria mirifica*, *Butea superba* and *Mucuna macrocarpa*

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Abstract: In this study, crude extracts of *Pueraria mirifica*, *Butea superba* and *Mucuna macrocarpa* were prepared using sequential extraction with three different solvents (hexane, ethyl acetate and methanol). The extracts obtained were then used to test for their antimicrobial activity by the disc diffusion method, which showed that, against a wide range of Gram-positive and Gram-negative bacteria, only the *P. mirifica* extract obtained with ethyl acetate exhibited antimicrobial activities. The minimum inhibitory concentration (MIC) of the extract was also determined with values between 15 and 50 mg/ml depending on the microbes tested. Thin layer chromatography (TLC) was subsequently used to separate the chemical constituents of the extract. When tested against *B. cereus*, there were only two bands which showed anti-*B. cereus* activity. Additionally, the crude extracts of *P. mirifica*, *B. superba* and *M. macrocarpa* were analysed for some antioxidant compounds using HPLC. Our results showed that all the extracts contained daidzin, genistin, daidzein and genistein, all of which were present in the highest amounts (0.045, 0.037, 0.049 and 0.060 % respectively) in the ethyl acetate extract of *P. mirifica*.

Keywords: antimicrobial activity, antioxidants, Kwao Krua, *Butea superba*, *Mucuna macrocarpa*, *Pueraria mirifica*

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Introduction

Kwao Krua is a generic name of a group of indigenous Thai medicinal plants in the family Leguminosae that have been widely used for a long time by the Thai people. Generally, the term is used for three different plant species, viz. white Kwao Krua (*Pueraria mirifica* Airy-Shaw & Suvatabandhu), red Kwao Krua (*Butea superba* Roxb.), and black Kwao Krua (*Mucuna macrocarpa* Wall.) [1]. There is also a description recorded for a *mor* (grey) Kwao Krua, but its exact identity remains unclear at present. It is suggested [2] that all these plants have rejuvenating properties, although this is found to be highly dependent on the type of Kwao Krua, i.e. black, red or white, and that they are to be taken only by the elderly. In addition to the rejuvenating properties, there are several phytoestrogenic compounds which include deoxymiroestrol, miroestrol, puerarin, daidzein, genistein, kwakhurin and other isoflavonoids, which can be used in medical applications due to their female hormone-like activity. Their estrogenic activity has also been exploited in many commercial products for breast enlargement and body firming [3,4].

Most of the investigations of Kwao Krua plants reported have focused upon their estrogenic and antioxidant activities. Although there have been many claims reporting the antimicrobial properties of these plants, they are still not conclusive due to the lack and/or limitation of scientific documentation. Hakamatsuka et al. [5] reported that *P. lobata* produces pterocarpan phytoalexins, namely tuberosin and glycinol, which prevent microbial attacks. A discovery was made in *B. superba* of a new bioactive flavonol glycoside and revealed its antimicrobial activity against plant pathogenic fungi and Gram-positive and Gram-negative bacteria [6]. There is, however, only one document describing the antimicrobial activity of *M. macrocarpa* [7]. This current study has been performed to investigate the antimicrobial properties of these Kwao Krua plants. The content of some antioxidant compounds in the crude extracts of these plants was also determined.

Materials and Methods

Plant materials

Tubers of *P. mirifica* and *B. superba* (Figure 1) were collected from Chiang Muan, Phayao Province, while *M. macrocarpa* was collected at the same time from Doi Tung, Chiang Rai Province in February 2004. The specimens were then authenticated and kept as voucher specimens Nos. MFLU-307, MFLU-310, and MFLU-311 respectively at Mae Fah Luang University Herbarium.

Microorganisms used

All the microbes used in the experiments were purchased from the Microbiological Resources Centre of the Thailand Institute of Scientific and Technological Research and kept as stock cultures at the Microbiology Laboratory in Mae Fah Luang University (Table 2). For routine culture and maintenance, the bacteria were grown on nutrient agar (NA) or in nutrient broth (NB) at 37°C. Yeasts were grown on the yeast malt agar (YMA) or in broth (YMB) at 30°C. For long term storage, all the microbes were kept either in a slant culture at 4°C or in glycerol stock at -20°C.

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Figure 1. Kwao Krua tubers: left, *P. mirifica*; middle, *B. superba*; right, *M. macrocarpa*

Preparation of Kwao Krua extracts

In this study, sequential extraction was performed as described by Canales et al. [8]. Initially, tuber samples were cut into small pieces, dried at 60°C for three days, and ground into a fine powder. The powdered samples (0.5 kg) were then placed in a closed plastic bag and stored in stainless steel coolers in a desiccator at ambient temperature (~28-30°C) until use.

Three separate extracts from each powdered Kwao Krua were made using 3 L each of hexane, ethyl acetate and methanol in that order. Each extract was left to stand for three days before filtering and then concentrated under reduced pressure at 45ºC using a vacuum rotary evaporator. The residue was dried and frozen with a freeze dryer (Flexi-DryTM, FTS Systems, USA) until a constant weight was obtained (Figure 2).

Antimicrobial assay

The microbial stock samples were cultured in 100 ml of NB or YMB and incubated at 37°C (for bacteria) or 30°C (for yeast) for 20-24 h in a shaking incubator. Each microbial cell sample was then inoculated onto agar plates. Sterile filter papers (diameter 6 mm, No. 3, Whatman, UK) were dipped in solutions of the three different sets of Kwao Krua extracts and placed on the surface of the inoculated agar plates. The plates were then incubated at either 37°C (for bacteria) or 30°C (for yeast) for 24 h. Each antimicrobial assay was carried out in triplicate by observing the clear zone formed on each plate. The antimicrobial activity was observed as the clear zone diameter seen on the plates and recorded in millimeters. Controls were made of pure hexane, ethyl acetate and methanol. The minimum inhibitory concentration (MIC) was determined from the extract samples, which were prepared at various concentrations (5-50 mg/ml). A 15 µl aliquot of each prepared extract was dropped on the sterile filter paper and placed onto the agar plate containing the tested microbes and the plate placed in an incubator at 37°C or 30°C for 24 h. This was also performed in triplicate. The presence of a clear zone at the lowest concentration was expressed as the MIC value.

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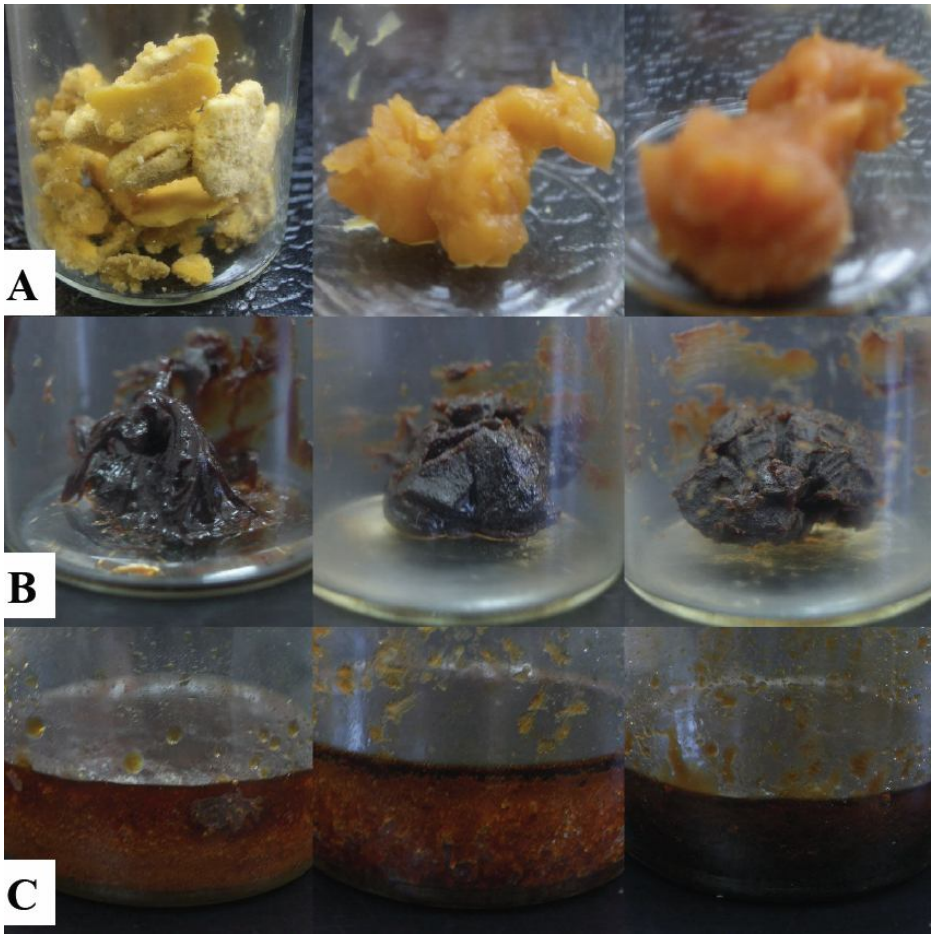

Figure 2. The appearance of Kwao Krua extracts derived from sequential extraction. Rows: A, hexane; B, ethyl acetate; C, methanol. Columns: left, *P. mirifica*; middle, *B. superba*; right, *M. macrocarpa*.

Determination of antimicrobial compounds using thin layer chromatography (TLC)

TLC technique was used to separate the chemical components present in the Kwao Krua extracts. The TLC plates (Silica gel aluminium plate GF254, J. T. Baker, USA) 20 cm x 20 cm in size were used. The extracts and the standard compounds (1 mg/ml of daidzein and genistein) were applied onto the plate, which was then placed in a chamber saturated with hexane and ethyl acetate (6:4) The developed chromatogram was placed under a 254 nm UV light. Each fraction was marked, scraped, dissolved in ethyl acetate (1 ml) and the resulting solution used for the antimicrobial assay above.

Analysis of antioxidant compounds

The analysis of the antioxidant compounds was based on that of Klump et al. [9]. A 100 mg sample of each Kwao Krua extract was dissolved in 40 ml of 80% methanol. The resulting suspension was then placed in an ultrasonic washer (Transsonic 700, Elma) for 5 min and then 2 M NaOH (3 ml) was added and the mixture incubated for 1 min. Subsequently, 1 ml of acetic acid was added to the incubated suspension and the solution was further incubated for 1 min. Finally, the supernatant of the prepared sample was filtered using a syringe filter (Chorm Tech., USA) and thus ready for analysis by HPLC (Water 2695 System). The conditions used were as follows: column, reverse-phase C-18; detector, UV 260 nm; mobile phase, water:methanol:acetic acid (88:10:2) and methanol:acetic acid

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(98:2). Standard antioxidants, namely daidzin, genistin, daidzein and genistein (Sigma-Aldrich Co., USA) were also prepared and used for this analysis.

Results and Discussion

Kwao Krua extracts

After evaporation and freeze-drying, the crude extracts of Kwao Krua were obtained as either a thickened solution or in solid form (Figure 2). The per cent yield of the extract (Table 1) was found to be highly dependent on the type of solvent used. As illustrated in Table 1, the per cent yields of the methanol extracts were the highest.

Table 1. Physical appearance and percent yield of Kwao Krua extracts

Kwao Krua extracts	Appearance	% Yield (w/w)
Hexane extract		
P. mirifica	White-yellow solid	0.21
B. superba	Brown solid	0.13
M. macrocarpa	Brown solid	0.29
Ethyl acetate extract		
P. mirifica	Dark brown solid	0.58
B. superba	Dark brown solid	0.36
M. macrocarpa	Dark brown solid	0.34
Methanol extract		
P. mirifica	Dark brown liquid	11.37
B. superba	Dark brown liquid	15.34
M. macrocarpa	Dark brown liquid	11.46

Antimicrobial activity of Kwao Krua extracts

All Kwao Krua extracts were preliminarily evaluated for their antimicrobial property using the disc diffusion method. In this study, seventeen species of microbes were selected including those that cause food poisoning (i.e. *B. cereus*, *E. coli* and *S. aureus*) and an opportunistic fungal pathogen (i.e. *C. albicans*). The results of this antimicrobial activity test are presented in Table 2.

It was found that only the ethyl acetate extract of *P. mirifica* exhibited antimicrobial activity. The extract was active against various kinds of Gram-positive and Gram-negative bacteria. For yeasts, the extract was able to inhibit *S. cerevisiae* but not *Candida* species. Interestingly, the extract was capable of inhibiting all the Gram-positive bacteria used in this study. However, for Gram-negative bacteria, it could not inhibit *A. faecalis* and *E. aerogenes*.

Further experiment was then carried out using different concentrations of *P. mirifica* ethyl acetate extract. The concentrations were prepared in the range of 25-100 mg/ml and the results are shown in Table 3. It can be seen that the crude extract (100 mg/ml) was most effective when used against *S. lactis*, giving a maximum clear zone of 11.17 ± 0.29 mm. The MIC experiment was then

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performed with various prepared concentrations of the crude extract and the MIC value was expressed as the lowest concentration of the extract that still exhibits antimicrobial activity. The concentrations used in this study were between 15-50 mg/ml and the results are shown in Table 4.

Table 2. Antimicrobial activity of Kwao Krua extracts (100 mg/ml) determined by the disc diffusion method

Microorganisms	C	*P. mirifica*			*B. superba*			*M. macropana*		
		H	E	M	H	E	M	H	E	M
Gram-positive bacteria										
Bacillus cereus TISTR 687	-	-	+	-	-	-	-	-	-	-
B. subtilis TISTR 008	-	-	+	-	-	-	-	-	-	-
Micrococcus luteus TISTR 884	-	-	+	-	-	-	-	-	-	-
Staphylococcus aureus TISTR1466	-	-	+	-	-	-	-	-	-	-
S. epidermidis TISTR 518	-	-	+	-	-	-	-	-	-	-
Streptococcus feacalis TISTR 459	-	-	+	-	-	-	-	-	-	-
Strep. lactis TISTR 457	-	-	+	-	-	-	-	-	-	-
Gram-negative bacteria										
Alcaligenes faecalis TISTR 038	-	-	-	-	-	-	-	-	-	-
Enterobacter aerogenes TISTR 1468	-	-	-	-	-	-	-	-	-	-
Escherichia coli TISTR 780	-	-	+	-	-	-	-	-	-	-
Proteus mirabilis TISTR 100	-	-	+	-	-	-	-	-	-	-
Pseudomonas fluorescens TISTR 358	-	-	+	-	-	-	-	-	-	-
Salmonella typhimurium TISTR 292	-	-	+	-	-	-	-	-	-	-
Serratia marcescens TISTR 1354	-	-	+	-	-	-	-	-	-	-
Yeasts										
Candida albicans TISTR 5239	-	-	-	-	-	-	-	-	-	-
C. utilis TISTR 5001	-	-	-	-	-	-	-	-	-	-
Saccharomyces cerevisiae TISTR 5049	-	-	+	-	-	-	-	-	-	-

Notes: i) C = pure solvent used as control for each extract; H = hexane extract; E = ethyl acetate extract; M = methanol extract

ii) + = presence of inhibition zone; - = no inhibition zone

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Table 3. Antimicrobial activity of *P. mirifica* extract (ethyl acetate fraction) determined by the disc diffusion method. Data shown are mean ± SD (mm) from three separate experiments.

Microorganisms	C	Clear zone diameter (mm)			
		25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
B. cereus	-	7.00 ± 0.50	7.83 ± 0.29	8.83 ± 0.29	10.50 ± 0.50
B. subtilis	-	-	7.00 ± 0	7.16 ± 0.29	7.83 ± 0.29
E. coli	-	7.16 ± 0.76	7.50 ± 0.50	8.16 ± 0.76	9.67 ± 0.58
M. luteus	-	7.00 ± 0	8.16 ± 0.29	8.83 ± 0.29	10.67 ± 0.76
Pro. mirabilis	-	7.00 ± 0	7.83 ± 0.29	8.33 ± 0.29	10.17 ± 0.29
Ps. fluorescens	-	-	7.33 ± 0.58	8.00 ± 0.50	9.50 ± 0.50
Sac. cerevisiae	-	7.00 ± 0	8.00 ± 0	8.50 ± 0.50	10.17 ± 1.15
Sal. typhimurium	-	-	7.00 ± 0	7.83 ± 0.29	8.67 ± 0.58
Ser. marcescens	-	-	8.00 ± 0	8.00 ± 0	9.33 ± 0.58
S. aureus	-	-	7.83 ± 0.76	8.33 ± 0.76	9.33 ± 0.76
S. epidermidis	-	7.00 ± 0	8.33 ± 0.58	8.67 ± 0.76	10.33 ± 0.58
Strep. feacalis	-	7.00 ± 0	8.00 ± 0	8.17 ± 0.29	9.67 ± 2.02
Strep. lactis	-	7.00 ± 0	8.67 ± 0.76	9.17 ± 0.76	11.17 ± 0.29

Notes: C = control; - = no inhibition zone

Table 4. MIC of *P. miricifa* extract (ethyl acetate fraction) for various tested microbes

Microorganisms	MIC (mg/ml)	Clear zone (mm)
Gram-positive bacteria		
B. cereus	20	7.0
M. luteus	20	7.0
S. aureus	50	7.0
S. lactis	15	7.0
Gram-negative bacteria		
P. fluorescens	50	7.0
P. mirabilis	15	7.0
S. *typhimurium*	45	6.5

It should be noted that the antimicrobial activity of the extract seemed to be more effective on Gram-positive bacteria than the Gram-negative ones. This is probably due to the difference in the cell wall structure of these bacterial groups. Gram-negative bacteria have an outer phospholipid membrane carrying the lipopolysaccharide structure, thus making the cell wall impermeable to lipophilic solutes. Gram-positive bacteria only have an outer peptidoglycan layer, which is not an effective permeability barrier, and thus are more susceptible than Gram-negative bacteria [10].

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There are a few reports of some antimicrobial phytoalexins in the Genus *Pueraria*. These include pterocarpans phytoalexins, namely tuberosin and glycinol, which are found in *P. lobata* [5]. These compounds were synthesised from the basic isoflavones, such as daidzein and genistein, which are also found in *P. mirifica* [11]. Furthermore, Verdrengh et al. [12] reported that genistein can inhibit *B. cereus*, *Helicobacter pylori*, and *S. pasteurianus*, whereas daidzein inhibits the growth of *S. aureus*. In addition, Yadava and Reddy [6] reported the antimicrobial activity of *B. superba* whose stem was used to prepare an extract which was tested for its antimicrobial activity. It was found that this extract was able to inhibit several bacterial and fungal species due to the presence of a novel active compound, chemically called 3,5,7,3',4'-pentahydroxy-8-methoxy-flavonol-3-O-beta-D-xylopyranosyl(1-2)-alpha-L-rhamnopyranoside. However, our study has shown a different result, which could most likely be due to different parts of *B. superba* being used as well as different methods of extraction.

Analysis of bioactive compounds using TLC

The components of the *P. mirifica* extract (ethyl acetate fraction) were separated by TLC and each fraction present under UV was used to test for anti-*B. cereus* activity. Of all the bands appearing on the TLC chromatogram, the ones with R_f values of 0.60 and 0.51 exhibited antimicrobial activity against *B. cereus*. Previous work had suggested the antimicrobial activity of daidzein and genistein against *B. cereus* [12]. According to the TLC analysis, however, our results have indicated that the bioactive compounds of *P. mirifica* extracts are not daidzein and genistein (data not shown). Besides, the standard daidzein and genistein used for antimicrobial purposes did not show any inhibition zone in this study. On the other hand, this might be owing to low amounts of both compounds used. As noted by Mbukwa et al. [13], the isoflavonoid genistein at 100 µg was active against *E. coli* while daidzein at this loading was not.

Antioxidants in Kwao Krua extracts

The presence of antioxidants in Kwao Krua extracts was determined by HPLC, which was performed with daidzin, genistin, daidzein, and genistein being used as chemical standards. The results of this analysis are presented in Table 5. Interestingly, only the *P. mirifica* ethyl acetate extract had the highest amounts of all four compounds among all the Kwao Krua extracts studied.

Generally, plants in the genus *Pueraria* have been known to contain isoflavones, especially in *P. lobata* and *P. thomsonii* [14-16]. In 1999, Zeng [17] reported that the tuber of *P. lobata* collected from Nanchang, Jiangxi, China in February 1995 contained 0.46% daidzin and 0.02% daidzein, while Lian et al. [18] found that the tuber of *P. thomsonii* collected from Pingnan, Guangxi, China in February 1989 contained 0.10% daidzin and 0.02% daidzein. Although *P. lobata* and *P. thomsonii* contain more daidzin than *P. mirifica*, the content of daidzein in *P. mirifica* seems to be higher than in either of those two plant species.

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Table 5. Some antioxidant compounds in Kwao Krua extracts (by HPLC). Data shown are in the unit of % (w/w).

Kwao Krua extracts	Daidzin	Genistin	Daidzein	Genistein
Hexane				
P. mirifica	-	-	0.003	0.054
B. superba	-	-	-	0.055
M. macrocarpa	-	-	-	0.054
Ethyl acetate				
P. mirifica	0.045	0.037	0.049	0.060
B. superba	-	0.010	0.005	0.015
M. macrocarpa	-	-	-	0.016
Methanol				
P. mirifica	0.019	0.011	-	0.054
B. superba	-	0.005	0.001	0.054
M. macrocarpa	-	-	-	0.054

Conclusions

This present study was performed to shed light on whether Kwao Krua plants exhibit any antimicrobial activity on account of the current scarcity of information on the subject. Our results clearly show that of those studied only the *P. mirifica* extract obtained with ethyl acetate exhibits antimicrobial activity against various Gram-positive and Gram-negative bacteria. The same extract is also the one that contains all four antioxidant compounds, viz. daidzin, genistin, daidzein and genistein, and in the highest amounts. Further experimentation on the bioactive compounds in this plant extract is expected to be carried out in the near future for its possible medicinal use.

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Effect of indigenous arbuscular mycorrhizal fungi on some growth parameters and phytochemical constituents of *Pogostemon patchouli* Pellet

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Abstract: Patchouli (*Pogostemon patchouli* Pellet) is an important aromatic crop cultivated for its essential oil used in cosmetics. arbuscular mycorrhizal (AM) fungi is known to modify several aspects of plant physiology and phytochemical constituents. Hence, a study was conducted on the efficacy of certain AM fungi in the improvement of some growth parameters and content of some phytochemical constituents in the leaves of *P. patchouli*. Patchouli seedlings were raised in soil inoculated with isolates of seven indigenous AM fungi, viz. *Acaulospora scrobiculata*, *Gigaspora margarita*, *Glomus aggregatum*, *G. geosporum*, *G. mosseae*, *Sclerocystis pakistanika* and *Scutellospora heterogama*. Seedlings raised in the presence of AM fungi generally showed an increase in growth, nutritional ingredients (sugars, N, P, K, Zn, Ca and Mn), total chlorophyll, and secondary metabolites in the leaves of patchouli compared to those from seedlings grown in the absence of AM fungi, the extent of increase, however, being varied with the AM fungi species. Furthermore, it was found that the phosphorus concentration was positively correlated with all growth parameters and content of phytochemical constituents (except essential oil), and that *G. aggregatum* seemed to be the best AM symbiont for the patchouli plant used in this experiment.

Keywords: AM fungi, *Pogostemon patchouli*, patchouli, growth parameters, phytochemical constituents

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Introduction

Patchouli (*Pogostemon patchouli* Pellet. Family: Lamiaceae) is an important aromatic crop native to Phillippines. It is cultivated for its essential oil used in cosmetics. Production of patchouli oil in India is negligible (about 100-150 kg/year), as against the global production of around 700-800 tonnes/year. Presently, India is importing over 200 tonnes of the oil from Indonesia, Malaysia and Singapore [1]. Hence, there is a good scope for growing patchouli as a main crop or intercrop with other plantation crops. The use of microbial inoculants is playing an important role in sustainable agriculture. Utilisation of mycorrhizal biofertilisers in the cultivation of medicinal and aromatic plants is of recent interest. Arbuscular mycorrhizal (AM) fungi have been used to enhance the plant growth and yield of medicinal crops and to help maintain good soil health and fertility that contributes to a greater extent to a sustainable yield and good quality of the products [2]. AM fungi can also alter plant-water relation and response to drought [3]. Due to their ability to increase nutrient uptake and water transport, AM fungi are being frequently used in sustainable agriculture [4]. Though these fungi are not host specific, recent studies [5,6] have clearly brought out host preference in AM fungi, thus emphasising the need for selecting efficient AM fungi for inoculating a particular host [7-9]. The productivity of many plants is dependent on the formation of AM fungi. However, there have been only few attempts to study the impact of AM inoculation on patchouli plant [1,10].

As a result of this symbiotic association between AM fungi and host plants, phosphorus content also has an effect on phytochemical constituents and growth parameters in plants [11-13]. Phosphorus plays an important role as an energy carrier during photosynthesis [14]. Therefore, AM fungi may function as a metabolic sink causing basipetal mobilisation of photosynthesis to roots, thus providing a stimulus for greater photosynthetic activity [13]. AM fungi, as obligate symbionts, also depend for their growth and activity on the supply of carbon compounds from the photosynthetic partner [15,16]. AM symbiosis can cause an important carbohydrate gain in the host plant and up to 20% of total photoassimilate substances can be transferred to the fungal partner [17]. Photosynthetic activity and carbohydrates, which are the photoassimilate substances, are very important in terms of parameters that explain the physiological activity of the plants mentioned above. For this reason, this study aims to screen for efficient AM fungi for patchouli plant and also to study the effect of the association of different indigenous AM fungi on some growth parameters, viz. reducing and total sugars, chlorophyll level, and content of secondary metabolites, viz. total phenols, ortho-dihydroxyphenols, alkaloids, flavonoids, tannins, saponins and essential oil in the leaves of *P. patchouli*. The relationship between the phosphate content and these parameters in the mycorrhizal patchouli plant is also investigated.

Materials and Methods

Cultivation of plants

This investigation was carried out under nursery condition in a glasshouse. Seedlings of *Pogostemon patchouli* were obtained from the nursery unit of Department of Horticulture at Tamil

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University, India. The identity of the plant was ascertained [18] and seedlings through stem cuttings (uniform 5-cm length) were raised on sterile sand-soil (1:1) mix. Three-week-old seedlings were transplanted to pots (24-cm dia.) containing 5 kg of sand-soil (1:3) mix which was classified as fine entisol, isohyperthermic kanhaplustalfs. The soil pH was 7.4 (1:10 soil-to-water ratio) and it contained 3.2 μg available phosphorus (extractable with NH_4F + HCl) and an indigenous AM fungal population at 60 spores/50g of soil. The AM fungal species (*Acaulospora scrobiculata, Gigaspora margarita, Glomus aggregatum, G. geosporum, G. mosseae, Sclerocystis pakistanica* and *Scutellospora heterogama*) used in this study were isolated from rhizosphere soil of patchouli plants. These AM fungal species were isolated by wet-sieving and decanting technique [19]. The species-level identification of different AM fungal species was done following the keys provided by Schenck and Perez [20]. These fungi were multiplied using sterilised sand and soil mix(1:1v/v) as substrate and guinea grass (*Panicum maximum* Jacq.) as host. After 90 days of growth, shoots of guinea grass were severed and the substrate containing hyphae, spores and root bits was air-dried and used as inoculum. The inoculum potential (IP) of each culture was estimated by adopting the most probable number (MPN) method as outlined by Porter [21]. The soil in each pot was mixed with this inoculum so as to maintain an initial IP of 12,500 per pot. One set of plants without inoculation was used as control. Each treatment with 5 replications was performed in the glasshouse and watered regularly so as to maintain the field capacity of the soil. Ruakura plant nutrient solution without phosphate was prepared [22] and then added to the pots at 50 ml per pot once every 20 days.

Harvesting and analysis of plants

Seventy-five days after transplanting, the plants were harvested for determination of the mycorrhizal status, growth response, nutritional status, and some physiological and phytochemical constituents. Plant height was measured from soil surface to the growing tip of the plant. Dry biomass was determined after drying the plant sample at 60^{o}C to constant weight in a hot-air oven. The root system was removed and assessed for AM fungal infection by grid-line intersect method [23] after clearing the roots with 10% KOH and staining with trypan blue (0.02%) as described by Phillips and Hayman [24]. Soil sample (100 g) was collected from each pot and subjected to wet-sieving and decantation method as outlined by Gerdemann and Nicolson [19] to estimate the population of spores. The essential oil content in the leaves of mycorrhizal and non-mycorrhizal plants were determined by hydro-distillation method by using Clevenger-type apparatus. Phosphorus, nitrogen, potassium and calcium content of the plant tissue were determined by employing the vanadomolybdophosphoric acid [25], micro-Kjeldahl [26], flame photometric and versenate titration method [25] respectively. Atomic absorption spectrophotometry was employed to estimate zinc and manganese content in the plant samples using respective hollow cathode lamps.

The content of chlorophylls in the leaves were determined spectrophotometrically [27]. The total and reducing sugar content in the leaves was estimated by employing Nelson-Somogyi reaction using glucose as standard [28]. The determination of total phenols and ortho-dihydroxyphenols was

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done using Folin-Ciocalteu reagent as outlined by Farkas and Kiraly [29] and Arnow's reagent with catechol as standard [30] respectively. Aluminium chloride colorimetric method was used for determination of flavonoids with quercetin as standard [31]. Alkaloid content was estimated by extraction and precipitation by ammonium hydroxide [32]. Tannin was determined by absorbance measurement of its complex with ferric chloride and potassium ferrocyanide using tannic acid as standard [33]. Saponin was estimated by extraction with 20% ethanol with subsequent solvent fractionations as outlined by Zakaria [33].

Statistical analysis

The generated data were subjected to statistical analysis by completely randomised block design and the means were separated by Duncan's Multiple Range Test (DMRT). The data were also analysed by linear regression and analysis of variance using SAS, and the means were compared between treatments by the t-test.

Results and Discussion

Growth response, nutritional status and physiological parameters

The responses of patchouli plants to inoculation with different AM fungi were found to vary. Mycorrhizal inoculation resulted in a significant increase in height, biomass, and nutrient content of the plants (Tables 1-2). Those inoculated with *Glomus aggregatum* showed highest increase in all growth parameters and nutritional components, followed by *Glomus mosseae*. However, *S. pakistanika* and *A. scrobiculata* did not show any significant differences from control (Tables 1-2). All mycorrhiza-treated plants were heavily colonised at the rate of between 48.5%-98.5% (Table 1). However, there was no positive correlation between plant growth parameters and mycorrhizal colonisation.

Earlier studies also showed the same trend for medicinal plants subjected to AM inoculation [5, 34-36] and these studies also indicated the host preference for the AM fungi. Bagyaraj and Varma [37], Chiramel et al.[6] and Rajeshkumar et al. [36] stressed the need for selecting efficient native AM fungi for plant species. The present study conducted with an objective of screening for efficient indigenous AM fungi for patchouli plant has also resulted in varied plant growth responses to different AM fungi. The extent of increase in phosphorus and zinc content in the plant varied among the fungi studied, with plants grown in the presence of *G. aggregatum* containing significantly highest content of these nutrients, followed by those grown in the presence of *G. mosseae*. Such a variation in the plant nutrient content in relation to fungal species for other medicinal plant species is well documented [6,35]. The enhancement in growth and nutritional status is also related to the per cent root colonisation apart from several soil and environmental factors.

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Table 1. Different native AM fungi and their influence on growth and oil content in leaf of *Pogostemon patchouli*

Treatment	Plant height (cm)		Plant dry weight (g/plant)			AM fungal colonisation in root (%)	Number of AM fungal spores / 100 g of soil
	Shoot	Root	Shoot	Root	Total		
Control (without AM fungi)	58.5[a]	24.2[a]	18.5[a]	12.6[a]	31.1[a]	0[a]	0[a]
Acaulospora scrobiculata	62.4[a]	26.2[a]	20.4[a]	13.2[a]	33.6[a]	50.0[a]	310[a]
Gigaspora margarita	65.8[b]	28.4[b]	21.2[b]	14.6[b]	35.8[b]	75.0[b]	420[b]
Glomus aggregatum	72.5[c]	29.5[c]	25.5[c]	16.4[c]	41.9[c]	98.5[c]	765[c]
Glomus geosporum	63.4[b]	26.5[b]	21.2[b]	14.4[b]	35.6[b]	72.5[b]	422[b]
Glomus mosseae	69.5[c]	27.2[c]	23.5[c]	16.1[c]	39.6[c]	80.0[b]	685[c]
Sclerocystis pakistanika	62.2[a]	25.8[a]	20.4[a]	13.0[a]	33.4[a]	48.5[a]	214[a]
Scutellospora heterogama	64.2[b]	26.4[a]	20.6[a]	13.2[a]	33.8[a]	52.5[a]	265[a]

Note: Means (n=5) in each column followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test.

The major concern in mycorrhizal technology for crop production is the existence of a great difference in the functional compatibility of AM fungi with medicinal crops [13,38,39]. In this study, inoculation with *G. aggregatum* and *G. mosseae* was found to be most effective in increasing the biomass (Table 1). AM fungi are known to improve plant growth and physiological parameters, mainly through uptake of phosphorus and other nutrients [13,40]. Bagyaraj et al.[41] and Lakshmipathy et al.[42] reported that different strains of AM fungi differ in the extent they increase nutrient uptake, physiological parameters and plant growth. Hence, some workers suggested the need for selecting efficient AM fungi that can be used for inoculating different plants [38]. In the present study, mycorrhizal inoculation enhanced the P, Zn, Ca and Mn content in the root and leaf of patchouli plant, which contributed to the enhanced growth of the plant (Table 2). Mycorrhizal treatment resulted in an increase in the number of spores in the rhizosphere soil and this was maximum in *G. aggregatum* (Table 1), followed by that in *Glomus mosseae*. It is well known that enhanced nutritional status of a plant is manifested in its improved growth [40].

The chlorophyll content in every inoculated plant was seen to be higher than that in uninoculated control (Table 3). In particular, the amount of chlorophylls a + b increased significantly ($P< 0.01$)(Table 3). The content of chlorophylls a, b and a + b increased in *Glomus aggregatum* treated plants by 14%, 12% and 18% respectively compared with those in uninoculated control plants. The

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Table 2. Influence of native AM fungi on N, P, K, Zn, Mn and Ca content in shoot and root of *Pogostemon patchouli*

Treatment	Nitrogen (g/plant)		Phosphorus (g/plant)		Potassium (g/plant)		Zinc (µg/plant)		Manganese (µg/plant)		Calcium (µg/plant)	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Control (without AM fungi)	0.285[a]	0.052[a]	0.204[a]	0.065[a]	0.282[a]	0.048[a]	0.216[a]	0.192[a]	0.016[a]	0.008[a]	29.0[a]	18.5[a]
Acaulospora scrobiculata	0.310[a]	0.058[a]	0.216[a]	0.072[a]	0.284[a]	0.052[a]	0.312[a]	0.210[a]	0.026[a]	0.009[a]	30.0[a]	19.2[b]
Gigaspora margarita	0.312[b]	0.062[b]	0.244[b]	0.085[b]	0.302[b]	0.054[b]	0.610[b]	0.312[b]	0.036[b]	0.010[b]	36.0[c]	19.8[c]
Glomus aggregatum	0.340[c]	0.064[c]	0.262[c]	0.098[c]	0.312[b]	0.054[b]	0.710[c]	0.352[c]	0.039[c]	0.012[c]	38.5[c]	20.0[c]
Glomus geosporum	0.308[b]	0.060[b]	0.248[b]	0.082[b]	0.286[a]	0.052[a]	0.610[b]	0.285[b]	0.028[b]	0.011[b]	29.5[a]	20.2[c]
Glomus mosseae	0.316[b]	0.062[b]	0.258[c]	0.092[c]	0.292[a]	0.049[a]	0.642[c]	0.314[b]	0.029[b]	0.011[b]	30.2[b]	19.2[b]
Sclerocystis pakistanika	0.305[a]	0.052[a]	0.218[a]	0.072[a]	0.284[a]	0.048[a]	0.314[a]	0.210[a]	0.024[a]	0.009[a]	29.5[a]	19.0[a]
Scutellospora heterogama	0.306[a]	0.052[a]	0.224[l]	0.074[a]	0.282[a]	0.049[a]	0.316[a]	0.214[a]	0.025[a]	0.009[a]	29.5[a]	18.8[a]

Note: Means (n = 5) in each column followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test.

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concentration of phosphorus was positively correlated with that of the chlorophylls in mycorrhizal plants, although it was not significant (Table5). The correlation coefficients were determined as r = 0.962, 0.753, and 0.867 for chlorophylls a, b and a + b respectively (Table 5). The partial correlations for all chlorophyll concentrations were also found to be non-significant ($P > 0.05$).

One of the most important indicators of physiological activity is the rate of photosynthesis, which is related to the chlorophyll content of plants. In this study, phosphorus and the chlorophyll content in all mycorrhizal plants were found to be higher than those in the uninoculated control, which indicated that the photosynthetic rate was improved by the AM fungi. Amelioration of the rate of photosynthesis and higher phosphorus level in leaves as a result of AM inoculation were also reported in other studies [13,43]. Since the photosynthetic process is known to be positively influenced by phosphorus, a positive correlation found in this study for phosphorus concentration in all mycorrhizal plants compared with that of uninoculated control of similar size also indicated its effect on photosynthesis. Furthermore, the effect of AM fungi on leaf morphology leading to an increase in the leaf area and leaf hydration is also probably partly caused by enhanced phosphorus level [44].

Table 3. Influence of native AM fungi on chlorophyll content and amount of sugars in the leaves of *P. patchouli*

Treatment	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Chlorophylls a + b (mg g^{-1})	Carbohydrate content	
				Reducing sugars (%)	Total sugars (%)
Control (without AM fungi)	$0.156^{a} \pm 0.014$	$0.332^{a} \pm 0.004$	$0.488^{a} \pm 0.012$	$0.28^{a} \pm 0.12$	$0.75^{a} \pm 0.015$
Acaulospora scrobiculata	$0.198^{b} \pm 0.012$	$0.398^{b} \pm 0.004$	$0.596^{b} \pm 0.014$	$0.48^{a} \pm 0.24$	$0.82^{b} \pm 0.025$
Gigaspora margarita	$0.202^{b} \pm 0.002$	$0.401^{b} \pm 0.004$	$0.603^{b} \pm 0.014$	$0.62^{b} \pm 0.42$	$0.85^{b} \pm 0.024$
Glomus aggregatum	$0.214^{c} \pm 0.002$	$0.406^{c} \pm 0.006$	$0.620^{c} \pm 0.014$	$0.96^{c} \pm 0.62$	$1.36^{c} \pm 0.045$
Glomus geosporum	$0.199^{b} \pm 0.002$	$0.402^{b} \pm 0.004$	$0.601^{b} \pm 0.012$	$0.84^{bc} \pm 0.54$	$0.92^{b} \pm 0.032$
Glomus mosseae	$0.212^{c} \pm 0.002$	$0.404^{c} \pm 0.002$	$0.616^{c} \pm 0.012$	$0.92^{c} \pm 0.62$	$1.24^{c} \pm 0.044$
Sclerocystis pakistanika	$0.196^{b} \pm 0.002$	$0.399^{b} \pm 0.002$	$0.595^{b} \pm 0.014$	$0.62^{b} \pm 0.42$	$0.86^{b} \pm 0.025$
Scutellospora heterogama	$0.198^{b} \pm 0.004$	$0.401^{b} \pm 0.002$	$0.599^{b} \pm 0.012$	$0.64^{b} \pm 0.42$	$0.88^{b} \pm 0.024$

Note: Means (n=5) in each column followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test.

The amounts of reducing and total sugars significantly differed in control and inoculated plants ($P < 0.05$) (Table 3). Moreover, the concentration of total phosphorus positively correlated with reducing and total sugar content in the treated plants (Table 5). The correlation co-efficient of reducing and total sugars were r = 0.982 and 0.304 respectively and found to be non-significant. Symbiotic interactions in AM association are based on the exchange of carbohydrates and mineral nutrients between the plant and the fungus [45]. It has been demonstrated, using mycorrhizal and non-mycorrhizal clover plants of comparable plant size and growth rate and with similar N and P content,

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that AM fungal colonisation stimulates the rate of photosynthesis sufficiently to compensate for the carbon requirement of the fungus and for growth reduction of the autotroph [46]. In this study, the content of carbohydrate compounds (reducing and total sugars) of mycorrhizal plants was also generally higher than that in the uninoculated control, and a positive correlation was determined between phosphorus concentration and all sugar content. Phosphorus plays the most important role during the breakdown of carbohydrates and synthesis of polysaccharides. In particular, phosphorus is very effective in the synthesis of starch from glucose [13]. As AM fungi increase the uptake of phosphorus, they may also increase the synthesis of carbon compounds [17]. Thus, it is seen that photosynthesis activity, increasing as a result of this symbiotic association, has a close relationship with the increase in the function of AM fungi.

Phytochemical constituents

The content of secondary metabolites (total phenols, ortho-dihydroxy phenols, alkaloids, flavonoids, tannins and saponins) in the leaves of patchouli plants were found to be significantly higher in those raised in soil inoculated with AM fungi (Table 4), with plants raised in the presence of *G. aggregatum* showing the most increase. (However, for all fungi tested there was no significant increase in essential oil content.) Such a variation in the phytochemical constituents in relation to fungal species for other medicinal plant species is also well documented [6,47]. The concentration of total phosphorus was also positively correlated with that of all secondary metabolites in the inoculated plants (Table 5). The correlation coefficients (r) of total phenols, ortho-dihydroxyphenols, flavonoids, alkaloids, tannins and saponins were 0.69, 0.62, 0.63, 0.92, 0.65 and 0.64 respectively (Table 5).

As AM fungi increase the uptake of phosphorus and other nutrients, they may also increase the synthesis of secondary metabolites. The increase in total phenols and ortho-dihydroxyphenols in inoculated plants could be attributed to the triggering of pathway of aromatic biosynthesis [48]. Krishna and Bagyaraj [49] reported an increase in phenols in the root of *Arachis hypogeae* colonised by *G. fasiculatum*. Hemalatha [50] also reported an increase in total phenols, ortho-dihydroxyphenols, flavonoids, alkaloids and tannins in the root and leaf of inoculated *Ocimum basilicum* and *Coleus amboinicus*. Codignola et al. [51] found that *Glomus versiforme* inoculated with *Allium porum* showed a higher level of phenols in both leaves and roots. Dhillion [52] confirmed host-mycorrhizal preference in some grassland species.

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Table 4. Influence of different native AM fungi on phytochemical constituents in the leaves of *P. patchouli*

Treatment	Total phenols (µg/g fresh wt.)	Ortho-di-hydroxy phenols (µg/g fresh wt.)	Alkaloids (µg/g dry weight)	Flavonoids (µg/g dry weight)	Tannins (µg/g dry weight)	Saponins (%)	Essential oil content in the leaves (%)
Control (uninoculated)	95.0[a]	64.2[a]	4.31[a]	3.12[a]	0.280[a]	0.160[a]	0.56[a]
Acaulospora scrobiculata	120.5[b]	72.3[b]	4.32[a]	3.22[a]	0.291[b]	0.172[b]	0.59[b]
Gigaspora margarita	124.2[b]	70.2[b]	4.38[b]	3.24[a]	0.294[b]	0.172[b]	0.62[c]
Glomus aggregatum	130.5[c]	85.4[c]	5.12[c]	3.76[c]	0.335[c]	0.192[c]	0.65[c]
Glomus geosporum	124.2[b]	74.2[b]	4.76[b]	3.62[b]	0.320[bc]	0.190[c]	0.59[b]
Glomus mosseae	128.5[c]	83.4[c]	4.92[c]	3.64[b]	0.332[c]	0.191[c]	0.62[c]
Sclerocystis pakistanika	121.4[b]	70.5[b]	4.38[b]	3.26[a]	0.295[b]	0.178[b]	0.58[a]
Scutellospora heterogama	122.5[b]	71.2[b]	4.32[a]	3.27[a]	0.294[b]	0.172[b]	0.57[a]

Note: Means (n = 5) in each column followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.

Table 5. Relationships between total phosphorus content and other organic constituents in *Glomus aggregatum* treated plants

Content	Y = a +	b.x +	$c.x^2$ +	$d.x^3$	R^2	r
Chlorophyll a [P3]	26.37 (-0.96 ns)	- 243.42 (0.96 ns)	748.24 (-0.96 ns)	- 763.02	0.92	0.962 NS
Chlorophyll b [P3]	- 5.47	56.73	- 180.94	190.67	0.54	0.753 NS
Total chlorophyll [P3]	- 0.87 (0.85 ns)	11.32 (- 0.84 ns)	- 160.98 (- 0.85 ns)	-	0.76	0.867 NS
Reducing sugars [P3]	10.72 (- 0.94 ns)	- 98.97 (0.95 ns)	324.42 (- 0.95 ns)	- 348.36	0.97	0.982 NS
Total sugars [P3]	36.12 (- 0.18 ns)	- 313.64 (0.17 ns)	796.52 (- 0.17 ns)	- 725.94	0.95	0.304 NS
Total phenols [P3]	- 7.21 (0.27 ns)	- 76.75 (- 0.28 ns)	- 256.28 (0.30 ns)	281.42	0.58	0.69 NS
Ortho di-hydroxy phenols [P3]	- 5.42 (0.24 ns)	64.75 (- 0.24 ns)	- 242.24 (0.24 ns)	- 264.42	0.44	0.62 NS
Flavonoids [P3]	- 19.90 (0.13 ns)	- 166.12 (- 0.10 ns)	- 421.54 (0.08 ns)	338.08	0.40	0.63 NS
Alkaloids [P3]	- 24.61 (0.8 ns)	238.52 (- 0.8 ns)	- 737.96 (0.8 ns)	750.35	0.84	0.92 NS
Tannins [P3]	14.12 (0.12 ns)	135.64 (- 0.12 ns)	- 418.56 (0.12 ns)	- 248.42	0.52	0.65 NS
Saponins [P3]	12.12 (0.10 ns)	124.42 (- 0.10 ns)	- 406.24 (0.10 ns)	- 236.46	0.48	0.64 NS

Note: P3 = Polynomial fit (degree 3)
NS = Correlation coefficient is not significant (P > 0.05).
ns = Partial correlation is not significant (P > 0.05).

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Conclusions

P. patchouli has been shown to exhibit varied responses to different AM fungi. Consideration of such factors as the growth parameters, nutritional status, and content of phytochemical constituents suggests that a specific relationship exists between a particular species of fungus and the plant. Giving weight to plant physiological growth parameters and phytochemical constituents but not neglecting the other parameters, *Glomus aggregatum* and *Glomus mosseae* seem to be the best and the next best fungus respectively for inoculating *P. patchouli* in the nursery in order to obtain healthy, vigorously growing seedlings that should perform better when planted in sandy loam soils, hence demonstrating and confirming that proper selection of efficient AM fungi for the right medicinal plant and environment is the key for their successful use in agriculture.

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