



Faculty of Resource Science and Technology

**Taxonomy, Phylogenetic Analyses and Pollination Biology of
Scaphochlamys (Zingiberaceae) of Borneo**

Ooi Im Hin

**Doctor of Philosophy
2018**

UNIVERSITI MALAYSIA SARAWAK

Grade: _____

Please tick (✓)

Final Year Project Report

Masters

PhD

☐☐☒

DECLARATION OF ORIGINAL WORK

This declaration is made on the2nd.....day of.....August 2018.....~~2016~~.

Student's Declaration:

IOOI IM HIN, 14010106, Faculty of Resource Science and Technology.....

(PLEASE INDICATE STUDENT'S NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled,Taxonomy, Phylogenetic Analyses and Pollination Biology of Scaphochlamys (Zingiberaceae) of Borneo..... is my original work. I have not copied from any other students' work or from any other sources except where due reference or acknowledgement is made explicitly in the text. nor has any part been written for me by another person.

2-8-2018

Date submitted

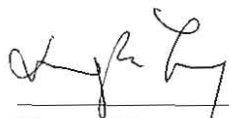

Name of the student (Matric No.)

OOI IM HIN (14010106)

Supervisor's Declaration:

I.....ASSOC. PROF. DR. WONG SIN YENG..... (SUPERVISOR'S NAME) hereby certifies that the work entitled,Taxonomy, Phylogenetic Analyses and Pollination Biology of Scaphochlamys (Zingiberaceae) of Borneo..... (TITLE) was prepared by the above named student, and was submitted to the "FACULTY" as a * partial/full fulfillment for the conferment ofDoctor of Philosophy (Plant Biology)..... (PLEASE INDICATE THE DEGREE), and the aforementioned work, to the best of my knowledge, is the said student's work

Received for examination by:


(Name of the supervisor)
(ASSOC PROF DR WONG SIN YENG)

Date: 21/8/18

I declare this Project/Thesis is classified as (Please tick (√)):

- ☐ **CONFIDENTIAL** (Contains confidential information under the Official Secret Act 1972)*
☐ **RESTRICTED** (Contains restricted information as specified by the organisation where research was done)*
☒ **OPEN ACCESS**

Validation of Project/Thesis

I therefore duly affirmed with free consent and willingness declared that this said Project/Thesis shall be placed officially in the Centre for Academic Information Services with the abide interest and rights as follows:

- This Project/Thesis is the sole legal property of Universiti Malaysia Sarawak (UNIMAS).
- The Centre for Academic Information Services has the lawful right to make copies for the purpose of academic and research only and not for other purpose.
- The Centre for Academic Information Services has the lawful right to digitise the content to for the Local Content Database.
- The Centre for Academic Information Services has the lawful right to make copies of the Project/Thesis for academic exchange between Higher Learning Institute.
- No dispute or any claim shall arise from the student itself neither third party on this Project/Thesis once it becomes sole property of UNIMAS.
- This Project/Thesis or any material, data and information related to it shall not be distributed, published or disclosed to any party by the student except with UNIMAS permission.

Student's signature _____

(Date)

(2-8-2018)

Supervisor's signature: _____

(Date)

21/8/18

Current Address:

3-7-2, Taman Jelutong Jaya, Lorong Penawar Satu, 11600 Georgetown, Pulau Pinang, Malaysia.

Notes: * If the Project/Thesis is **CONFIDENTIAL** or **RESTRICTED**, please attach together as annexure a letter from the organisation with the period and reasons of confidentiality and restriction.

[The instrument was duly prepared by The Centre for Academic Information Services]

Taxonomy, Phylogenetic Analyses and Pollination Biology of *Scaphochlamys*
(Zingiberaceae) of Borneo

Ooi Im Hin

A thesis submitted

In fulfilment of the requirements for the degree of Doctor of Philosophy (Plant Biology)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2018

DECLARATION

The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



Ooi Im Hin

14010106

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

ACKNOWLEDGEMENT

My greatest gratitude goes to my supervisor, Assoc. Prof. Dr. Wong Sin Yeng, for her constant guidance and continuous supports along this study. The constructive advices and supports in the management of living collections from Peter C. Boyce are gratefully thanked. The sharing of experiences from the fellow laboratory mates, Dr. Hoe Yin Chen and Dr. Low Shook Ling are highly valued. Thanks are due to the laboratory assistants, Shafri bin Semawi and Benedict Samling, for their technical supports. The field assistances and hospitality of all the local villagers and their families, Albert, Ambau ak Taging (Iduin), Arnold, Augustine, Bada, Bakong ak Frinsa, Bana, Davidson, Jeff, Jeland ak Kisai, Jepom ak Tisai, Neil, Sadi, Saga and Sunang ak Empin, are deeply appreciated. Gratitude goes to the staff of Penang Botanic Gardens, Maliga and Sivakumaran for their assistances. The researchers and naturalists, Brian Williams, Dr. Christopher Darling, John D. Mood, Dr. Marc Gibernau, Dr. Mark Newman, Michael Lo, Robert Tseu, Dr. Sam Yen Yen and Scott Hyndman are gratefully acknowledged for extending their help at various levels of this study. The infinite supports from my family and friends will never be forgotten. The contribution of R.I.P. Simon Kutuh ak Paru in previous plant collection records and the sacrifices of the plant casualties will always be remembered.

This study is funded by Ministry of Education Malaysia Niche Research Grant Scheme No. NRGS/1089/2013-(03) and MyBrain15 scholarship KPM(B) 811111075291. Recent fieldworks were carried out under permit no. NCCD.907.4.4.(JLD.12)-51 and park permit no. 121/2015 from Sarawak Forestry Department.

ABSTRACT

Scaphochlamys Baker is a genus of 44 species in the family Zingiberaceae distributed from Southern Thailand through Peninsular Malaysia until Sumatera and Borneo. Previous taxonomic and phylogenetic studies concentrated on the species from Peninsular Malaysia. The interactions between the diverse Zingiberaceae flowers and their pollinators are important. However, there was no documentation on the floral biology and pollination mechanism of *Scaphochlamys*. Therefore, in this study, an integrated approach (including taxonomy, phylogeny, floral and pollination biology incorporated with SEM and floral volatile organic compounds (VOCs) analyses) was employed to study the Bornean *Scaphochlamys*. As a result, 12 taxonomic novelties were described (*Scaphochlamys durga* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys graveolens* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys hasta* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys lanjakensis* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys lucens* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys multifolia* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys nigra* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys penyamar* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys pseudoreticosa* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys scintillans* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys stenophylla* I. H. Ooi & S. Y. Wong, sp. nov., and *Scaphochlamys uniflora* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov.). Several important morphologies were discussed. Six informal intrageneric taxonomic groups were proposed for Bornean *Scaphochlamys* species. A phylogeny of 26 *Scaphochlamys* species (36 accessions) with ITS and *trnK-matK* sequence data was generated. Most of the

Scaphochlamys species on Borneo formed a clade together with *Myxochlamys* A. Takano & Nagam., except *S. limiana* and *S. polyphylla* were clustered among *Scaphochlamys* species from Peninsular Malaysia. A one-day diurnal flowering mechanism was observed for 19 species of *Scaphochlamys*. All investigated Bornean *Scaphochlamys* are entomophilous xenogamy. Two types of traplining bees (*Amegilla* bees and Halictid bees) were concluded as the pollinators for eight Bornean *Scaphochlamys* species. The pollen size of 21 investigated *Scaphochlamys* species is within the range of 72.53 – 96.52 μm long \times 52.39 – 78.41 μm wide, spheroidal to elliptical, and more or less psilate. Three hundred and twenty one floral VOCs (mainly terpenes, terpenoids, aromatic compounds and fatty acid derivatives) were found in 17 species of *Scaphochlamys*. This study provides a comprehensive overview of the Bornean *Scaphochlamys* with the discovery of novel species, together with the phylogenetic relationship resolved, and interactions between plant-pollinator.

Keywords: Borneo, floral biology, morphology, phylogenetics, pollinator, *Scaphochlamys* (Zingiberaceae), taxonomy.

***Taksonomi, Analisis Filogenetik dan Biologi Pendebungaan Scaphochlamys
(Zingiberaceae) di Borneo***

ABSTRAK

Scaphochlamys Baker ialah salah satu genus dalam famili *Zingiberaceae* yang mengandungi 44 spesies tertabur dari Selatan Thailand ke Semenanjung Malaysia sehingga Sumatera dan Borneo. Kebanyakan penyelidikan taksonomi dan filogenetik sebelum ini hanya tertumpu kepada spesies-spesies di Semenanjung Malaysia. Interaksi antara pelbagai bunga *Zingiberaceae* dan agen pendebungaan adalah penting. Akan tetapi, masih belum ada laporan tentang biologi bunga dan pendebungaan untuk *Scaphochlamys*. Oleh yang demikian, untuk kajian ini, satu pendekatan secara integrasi (termasuk taksonomi, filogeni, biologi bunga dan pendebungaan, bersama-sama dengan SEM dan analisis sebatian organik meruap (VOCs) bunga) telah digunakan untuk mengkaji *Scaphochlamys* di Borneo. Sebagai keputusannya, 12 spesies baharu telah dilaporkan (*Scaphochlamys durga* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys graveolens* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys hasta* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys lanjakensis* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys lucens* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys multifolia* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys nigra* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys penyamar* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys pseudoreticosa* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys scintillans* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov., *Scaphochlamys stenophylla* I. H. Ooi & S. Y. Wong, sp. nov., dan *Scaphochlamys uniflora* I. H. Ooi, K. Meekiong & S. Y. Wong, sp. nov.). Ciri-ciri

morfologi yang penting juga dibincangkan. Enam kumpulan taksonomi tidak formal dalam genus dicadangkan untuk spesies-spesies Scaphochlamys di Borneo. Satu filogeni dengan data rangkaian ITS dan trnK-matK yang meliputi 26 spesies Scaphochlamys (36 siri rujukan) telah dijanakan. Kebanyakan spesies-spesies Scaphochlamys di Borneo tergolong dalam satu kumpulan dengan Myxochlamys A. Takano & Nagam., kecuali S. limiana dan S. polyphylla tergolong dengan spesies-spesies Scaphochlamys dari Semenanjung Malaysia. Perkembangan bunga dalam sehari pada siang hari telah diteliti untuk 19 spesies Scaphochlamys. Semua Scaphochlamys di Borneo yang diteliti merupakan xenogami entomofili. Dua jenis lebah 'traplining' (lebah Amegilla dan lebah Halictid) telah dikenalpasti sebagai agen pendebungaan untuk lapan spesies Scaphochlamys. Saiz debunga untuk 21 spesies yang dikaji adalah dalam rangkuman $72.53 - 96.52 \mu\text{m} \times$ selebar $52.39 - 78.41 \mu\text{m}$, bulat atau bujur, dan lebih kurang licin. Tiga ratus dan dua puluh satu VOCs bunga (kumpulan terpena, terpenoid, sebatian aromatik dan terbitan asid lemak) telah ditemui di dalam 17 spesies Scaphochlamys. Kajian ini menghasilkan pengetahuan secara keseluruhan tentang Scaphochlamys di Borneo dengan penemuan spesies-spesies baru, bersama-sama dengan hubungan filogenetik secara resolusi, dan interaksi antara tumbuhan-agen pendebungaan.

Kata kunci: *Borneo, biologi bunga, morfologi, filogenetik, agen pendebungaan, Scaphochlamys (Zingiberaceae), taksonomi.*

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
<i>ABSTRAK</i>	v
TABLE OF CONTENTS	vii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
CHAPTER 1: INTRODUCTION	1
1.1 Background	1
1.2 Problem Statements	3
1.3 Objectives	4
CHAPTER 2: LITERATURE REVIEW	5
2.1 Zingiberaceae	5
2.2 <i>Scaphochlamys</i>	6
2.2.1 Background	6
2.2.2 Taxonomic History	8
2.2.3 Morphology of <i>Scaphochlamys</i>	9
2.2.4 Taxonomic Description	13
2.3 Bornean <i>Scaphochlamys</i>	15
2.4 Phylogeny	15
2.4.1 Gene Regions	15
2.4.1.1 <i>trnK-matK</i>	15

2.4.1.2 Internal Transcribed Spacer (ITS)	16
2.4.2 Phylogeny of <i>Scaphochlamys</i>	17
2.5 Floral Biology of Zingiberaceae	20
2.5.1 Floral Morphology and Development	20
2.5.2 Pollination Biology	23
2.5.3 Palynology	25
2.5.4 Seed Morphology	26
2.6 Floral Scent	26
CHAPTER 3: MORPHOLOGY AND TAXONOMY	28
3.1 Introduction	28
3.2 Materials and Methods	29
3.3 Results and Discussion	30
3.3.1 Morphology	30
3.3.1.1 Element	30
3.3.1.2 Pulvinus	30
3.3.1.3 Flowering Sequence	32
3.3.1.4 Inflorescence	32
3.3.1.5 Bracts	32
3.3.1.6 Bracteoles	33
3.3.1.7 Flowers and Labella	34
3.3.1.8 Anther Spurs	34
3.3.1.9 Fruits	35
3.3.2 Taxonomy	35
3.3.2.1 Generic Delimitation	35

3.3.2.2 Taxonomic Groupings Based on Morphological Characters	35
3.3.2.3 Key to Species Groups in Borneo	36
CHAPTER 4: MOLECULAR ANALYSES	99
4.1 Introduction	99
4.2 Materials and Methods	99
4.2.1 Taxa Sampling	99
4.2.2 DNA Extraction and Polymerase Chain Reaction (PCR)	103
Amplification and Sequencing	
4.2.3 Sequence Alignment and Phylogenetic Analyses	103
4.3 Results and Discussion	105
4.3.1 Matrix Characteristics	105
4.3.2 Phylogenetic Analyses	106
4.3.2.1 ITS Region	106
4.3.2.2 <i>trnK-matK</i> Region	109
4.3.2.3 Combined Regions	113
4.3.3 Discussion	116
4.3.3.1 Phylogenetic Relationship	116
4.3.3.2 Phylogeography	119
CHAPTER 5: FLORAL BIOLOGY	122
5.1 Introduction	122
5.2 Materials and Methods	122
5.2.1 Study Localities	122
5.2.1.1 Anomala Group	122
5.2.1.2 Calcicola Group	123

5.2.1.3 Graveolens Group	123
5.2.1.4 Limiana Group	124
5.2.1.5 Petiolata Group	124
5.2.1.6 Other Taxa	126
5.2.2 Flowering Mechanism and Pollination Observation	129
5.2.2.1 Stigma Receptivity Test	130
5.2.2.2 Pollen Germination Test	131
5.2.2.3 Self-pollination/autogamy Test	131
5.2.2.4 Floral Microstructures	132
5.2.2.5 Pollen Morphology	132
5.2.2.6 Pollen, Pollinator and Stigma	133
5.2.2.7 Pollen Histochemistry	133
5.2.2.8 Pollen : Ovule Ratios	134
5.2.2.9 Natural Fruit Set Percentage	134
5.2.2.10 Floral Volatile Organic Compounds (VOCs) Analysis	135
5.3 Results and Discussion	137
5.3.1 Flowering Mechanism and Pollination Observation	137
5.3.1.1 Observation of Investigated Taxa	142
5.3.1.2 Interaction among the Plants and Insect Visitors	170
5.3.2 Stigma Receptivity Test	178
5.3.3 Pollen Germination Test	178
5.3.4 Self-pollination/autogamy Test	179
5.3.5 Floral Microstructures	179
5.3.6 Pollen Morphology	183

5.3.7 Pollen, Pollinator and Stigma	190
5.3.8 Pollen Histochemistry	194
5.3.9 Pollen : Ovule Ratios (P/O)	195
5.3.10 Natural Fruit Set Percentage	195
5.3.11 Floral Volatile Organic Compounds (VOCs) Analysis	199
CHAPTER 6: CONCLUSION AND RECOMMENDATIONS	233
6.1 Conclusion	233
6.2 Recommendations or Future Prospects	235
REFERENCES	237
APPENDICES	262

LIST OF TABLES

		Page
Table 4.1	The taxa included in the phylogenetic analyses	100
Table 4.2	The primers used in this study	104
Table 5.1	The population size, number of inflorescences, number of fruits and number of seeds of the investigated species	127
Table 5.2	The anthesis timing, stigma movement and floral odour of investigated taxa	143
Table 5.3	The insect visitors of investigated species with the visiting frequencies	173
Table 5.4	The pollen measurements, exine ornaments and starch measurements of the investigated species	184
Table 5.5	The numbers of pollen, numbers of ovules and the pollen : ovules (P/O) ratios of the investigated taxa	196
Table 5.6	The total number of flowers, number of fruits and the calculated fruit set percentage of all investigated taxa	197
Table 5.7	The mean relative peak areas (%) of the floral VOCs chemical composition analysed by using GC-MS with BP20 polar column in splitless mode	200
Table 5.8	The average scent emission ($\mu\text{g hr}^{-1}$) by the flowers of each investigated species according to the floral VOCs chemical composition analysed by using GC-MS with BP20 polar column in splitless mode.	214

LIST OF FIGURES

	Page
Figure 2.1 Morphology of <i>Scaphochlamys</i>	11
Figure 3.1 Morphological characters	31
Figure 3.2 <i>Scaphochlamys lanjakensis</i>	40
Figure 3.3 <i>Scaphochlamys penyamar</i>	43
Figure 3.4 <i>Scaphochlamys scintillans</i>	47
Figure 3.5 <i>Scaphochlamys calcicola</i>	51
Figure 3.6 <i>Scaphochlamys uniflora</i>	52
Figure 3.7 <i>Scaphochlamys graveolens</i>	56
Figure 3.8 <i>Scaphochlamys lucens</i>	60
Figure 3.9 <i>Scaphochlamys limiana</i>	63
Figure 3.10 <i>Scaphochlamys argentea</i>	69
Figure 3.11 <i>Scaphochlamys biru</i>	71
Figure 3.12 <i>Scaphochlamys durga</i>	74
Figure 3.13 <i>Scaphochlamys hasta</i>	78
Figure 3.14 <i>Scaphochlamys multifolia</i>	81
Figure 3.15 <i>Scaphochlamys nigra</i>	84
Figure 3.16 <i>Scaphochlamys petiolata</i>	88
Figure 3.17 <i>Scaphochlamys pseudoreticosa</i>	90
Figure 3.18 <i>Scaphochlamys reticosa</i>	93
Figure 3.19 <i>Scaphochlamys stenophylla</i>	94
Figure 3.20 <i>Scaphochlamys polyphylla</i>	96
Figure 4.1 Single best Maximum Likelihood (ML) tree based on ITS region	107

Figure 4.2	Bayesian majority-rule consensus tree based on ITS region	108
Figure 4.3	Single best Maximum Likelihood (ML) tree based on <i>trnK-matK</i> region	110
Figure 4.4	Bayesian majority-rule consensus tree based on <i>trnK-matK</i> region	111
Figure 4.5	Single best Maximum Likelihood (ML) tree based on combined regions (ITS + <i>trnK-matK</i>)	114
Figure 4.6	Bayesian majority-rule consensus tree based on combined regions (ITS + <i>trnK-matK</i>)	115
Figure 5.1	Inflorescence position	139
Figure 5.2	Inflorescence structure	140
Figure 5.3	<i>Scaphochlamys lanjakensis</i>	148
Figure 5.4	<i>Scaphochlamys penyamar</i> and <i>S. scintillans</i>	150
Figure 5.5	<i>Scaphochlamys calcicola</i>	151
Figure 5.6	<i>Scaphochlamys graveolens</i> and <i>Boesenbergia puchella</i>	153
Figure 5.7	<i>Scaphochlamys lucens</i>	154
Figure 5.8	<i>Scaphochlamys limiana</i> and <i>S. argentea</i>	156
Figure 5.9	<i>Scaphochlamys biru</i> and <i>S. durga</i>	158
Figure 5.10	<i>Scaphochlamys hasta</i> and <i>S. nigra</i>	159
Figure 5.11	<i>Scaphochlamys multifolia</i>	161
Figure 5.12	<i>Scaphochlamys petiolata</i>	163
Figure 5.13	<i>Scaphochlamys pseudoreticosa</i> and <i>S. reticosa</i>	164
Figure 5.14	<i>Scaphochlamys stenophylla</i>	166
Figure 5.15	<i>Scaphochlamys polyphylla</i> and <i>S. kunstleri</i> var. <i>kunstleri</i>	167
Figure 5.16	<i>Scaphochlamys kunstleri</i> var. <i>speciosa</i> and <i>Plagiostachys</i>	169

crocydocalyx

Figure 5.17	<i>Scaphochlamys malaccana</i>	171
Figure 5.18	<i>Boesenbergia parva</i>	175
Figure 5.19	Floral microstructures	180
Figure 5.20	Floral microstructures	181
Figure 5.21	Pollen morphology	187
Figure 5.22	Pollen morphology	188
Figure 5.23	Pollen morphology	189
Figure 5.24	Pollen morphology	191
Figure 5.25	Halictid bee	192
Figure 5.26	Halictid bee and the stigma	193

CHAPTER 1

INTRODUCTION

1.1 Background

Zingiberaceae Martinov is well-known as the ginger family with many important culinary, medicinal and ornamental species. It is the largest family in order Zingiberales which consists of 56 genera with between 1,075-1,370 species (Stevens, 2001). Zingiberaceae is distributed throughout all the tropical regions globally but it is most diverse in South East Asia (Kress et al., 2002; Larsen, 2005) and contains four subfamilies (Siphonochiloideae W. J. Kress, Tamijioideae W. J. Kress, Alpinioideae Link and Zingiberoideae Hasskarl) with six tribes (Zingibereae, Globbeae, Alpinieae, Riedelieae, Tamijieae and Siphonochideae; *sensu* Kress et al., 2002).

Scaphochlamys Baker is a genus of 44 described species in the Kaempferia Clade within tribe Zingibereae (Kress et al., 2002; Leong-Škorničková et al., 2011) and closely related to *Distichochlamys* M. F. Newman and *Myxochlamys* A. Takano & Nagam. (Takano & Nagamasu, 2007). Searle (2010) summarized a combination of morphological characters: leaf-sheath base pulvinate (Borneo only); bract arrangement spiral; flowering mode from base to apex; first bracteole keeled, arising opposite to bract; bracteole split to base; thecae with free basal spurs, to distinguish *Scaphochlamys* from other closely related genera. *Scaphochlamys* is distributed from Southern Thailand, through Peninsular Malaysia, to Northern Borneo and Sumatera but each single species is usually narrowly distributed with a high degree of endemism (Searle, 2010). In Borneo, there are 14

described species with five recently described novelties (Mas Izzaty et al., 2013; Ooi & Wong, 2014; Meekiong et al., 2015), of which all species are endemic.

Searle and Hedderson (2000) included five species (*S. biloba* (Ridl.) Holttum, *S. concinna* (Baker) Holttum, *S. kunstleri* (Baker) Holttum var. *rubra* Ridl., *S. obcordata* P. Sirirugsa & K. Larsen and *S. reticosa* (Ridl.) R.M. Sm., one Bornean species) in their phylogenetic investigation on tribe Hedychieae (*sensu* Burtt & Smith, 1972c) but only ITS region was employed. Two Peninsular Malaysia species, *S. kunstleri* and *S. lanceolata* (Ridl.) Holttum, were included in the phylogeny of tribe Zingibereae with ITS and *trnL-trnF* regions (Ngamriabsakul et al., 2004). For phylogenetic studies with ITS and *matK* regions, previously, only two *Scaphochlamys* species from Peninsular Malaysia, *S. biloba* and *S. kunstleri*, were included (Kress et al., 2002; Takano & Nagamasu, 2007; Leong-Škorničková et al., 2011). Within Bornean *Scaphochlamys*, until very recently, only seven species were included in the phylogenetic analyses with both ITS and *matK* regions (Sam et al., 2016).

Specht et al. (2012) stated that the ancestral pollination syndrome of Zingiberaceae is probably insect or bee pollination based on character mapping of developmental evolutionary floral and pollination biology datasets on Zingiberales phylogeny. In Borneo, Sakai et al. (1999; 2013) conducted a pollination study on the gingers (29 species in 11 genera) of Lambir Hills National Park, Sarawak, Malaysia and investigated the correlation of flower measurements and the pollinators. Three pollination guilds were reported, which are Halictid bee, *Amegilla* bee and Spiderhunter bird (Sakai et al., 1999; 2013). Aside from this, pollination by carpenter bee was reported for *Alpinia nieuwenhuizii* Val. in

Kinabatangan district, Sabah, Malaysia (Takano et al., 2005). More techniques were applied to inspect various aspects of Zingiberaceae species for better understanding about the plants and their ecological interactions, for examples, scanning electron microscopy (SEM) for pollen morphology (Theilade et al., 1993); pollen histology for investigating pollen nutrition and pollination systems correlation (Wang et al., 2004); and floral volatile organic compounds (VOCs) analysis for biochemical compounds in floral scent emission (B  z et al., 2011). So far, there is yet to be a single documentation on the pollination biology of *Scaphochlamys*.

1.2 Problem Statement

Although Zingiberaceae is a commonly known plant family, except those common domestic species, most of the highly endemic species are still understudied with very limited information available. For *Scaphochlamys*, Searle (2010) stated that owing to several reasons such as historical collections did not preserve well, ephemeral flowers and weakly written descriptions, the taxonomic revision was difficult and more novelties were expected from Borneo and Sumatera. The generic boundary is defined by a set of morphological characters in combination rather than any single key character (Searle, 2010). Thus, dealing with the taxonomic problem is crucial to provide a solid foundation for further scientific investigation to progress.

In Malaysia, Sam (2014) stated the confusion in the generic delimitation and relationship of *Scaphochlamys* and other close genera, which *Myxochlamys* is embedded within *Scaphochlamys* in phylogeny. For examples, the distichous bract arrangement in *Distichochlamys*, the 2-keeled first bracteoles and the grooved inner corolla tube in

Myxochlamys are present in *Scaphochlamys* as well. As the previous studies were concentrating in Peninsular Malaysia, the interspecific relationship among the species from Borneo is poorly known. So far, only half of the species were included in previous studies. Despite the fact that the species belonging to the order Zingiberales have high floral variability and complex pollination system (Kress & Specht, 2005), the pollination biology of most of the taxa including those from *Scaphochlamys* is still unknown.

With the sampling activities in this study, it was hypothesized that more novel species might be found because there were no intensive collecting carried out in Borneo previously. For phylogenetics, the intergeneric relationship of *Scaphochlamys* and other genera might not differ much from previous studies but the interspecific relationship of the species from Borneo would be better known. Furthermore, it was speculated that this floral biology study would provide the information on the pollination system of *Scaphochlamys* and some insight for the generic delimitation.

1.3 Objectives

This study aimed:-

- (1) to study the morphology and taxonomy of the *Scaphochlamys* species on Borneo;
- (2) to produce a phylogeny inferring the relationship of the Bornean *Scaphochlamys* species;
- (3) to document the pollination strategies of the investigated taxa;
- (4) to undertake floral scent analysis and link this into the pollination strategies of the investigated taxa.

CHAPTER 2

LITERATURE REVIEW

2.1 Zingiberaceae

Zingiberaceae comprises 56 genera with between 1,075-1,370 species which are divided into four subfamilies: Siphonochiloideae, Tamijioideae, Alpinioideae and Zingiberoideae (Stevens, 2001) with tribe Zingibereae, tribe Globbeae, and *Monolophus* (formerly *Caulokaempferia*; Mood et al., 2014) nested within Zingiberoideae, and tribe Alpinieae, tribe Riedelieae, and *Siliquamomum* nested within Alpinioideae. Tamijioideae are monospecific and Siphonochiloideae contain two genera (Kress et al., 2002). All subfamilies are well supported based on molecular and morphological data, but many speciose genera (e.g., *Alpinia* Roxb., *Amomum* Roxb., and *Curcuma* L.) have been shown to be paraphyletic and/or polyphyletic (*Amomum*: Harris et al., 2000; Xia et al., 2004 ; *Alpinia*: Rangsiruji et al., 2000; Zingibereae: Ngamriabsakul et al., 2004; *Etlingera* Giseke: Pedersen, 2004; *Globba* L.: Williams et al., 2004; Alpinioideae: Kress et al., 2005, 2007; *Curcuma*: Z ávesk áet al., 2012; Leong-Škorničková et al., 2015).

Zingiberaceae are an economically and ecologically important family of commelinid monocots with a centre of diversity in South-East Asia (Kress et al., 2002; Larsen, 2005). Zingiberaceae is easily separated from other Zingiberales families by possessing ligulate distichous leaves, flowers with a single dithecal stamen, and an often showy petaloid labellum formed from two or four staminodes (Simpson, 2010). The age of crown-group Zingiberaceae is more than 70 m.y. (Auvray et al. 2010).