Faculty of Resource Science and Technology

Systematics Studies of the Genus *Amomum* Roxb. (Zingiberaceae): Fruit Morphological Aspects

Nurul Izzati Binti Azman (45958)

Bachelor of Science with Honours

(Plant Resources Science and Management)

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UNIVERSITI MALAYSIA SARAWAK

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Systematics Studies of the Genus *Amomum* Roxb. (Zingiberaceae): Fruit Morphological Aspect

Nurul Izzati Binti Azman (45958)

This project is submitted in partial fulfillment of the requirement for degree of

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APPROVAL SHEET

Name of Candidate: Nurul Izzati Binti Azman

Student No: 45958

Title of Project: Systematics Studies of the Genus *Amomum* Roxb. (Zingiberaceae): Fruit Morphological Aspects.

(Dr. Qammil Muzzammil Abdullah @ Meekiong Kalu)

Supervisor

(Dr. Freddy Yeo Kuok San)

Coordinator

Plant Resource Science and Management Programme

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak.

DECLARATION

This project is done for the subject of STF 3015 Final Year Project 2 2016/2017. It is based on my original work except for quotation and citation which have been acknowledged. I declare that no portion of this research work had been submitted to support the application of other degree or qualification at any other universities or institutions of higher learning.

Nurul Izzati Binti Azman

Plant Resource Science and Management Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

Jun, 2017

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TABLE OF CONTENT

ABBREVIATION

aff.	Affinis (around)
c.	Circa (approximately)
cm	Centimetre
mm	Milimetre
sp.	Species
SAR	Acronym for Herbarium Department of Forest Sarawak
SEM	Scanning Electron Microscope
HUMS	Acronym for Herbarium Universiti Malaysia Sarawak
UNIMAS	Universiti Malaysia Sarawak
MP	Megapixel
LED	Light-emitting diode
USB	Universal Serial Bus

LIST OF TABLE

Page

LIST OF FIGURES

Systematics studies of the Genus *Amomum* Roxb. (Zingiberaceae) in Malaysia: Fruit Morphological Aspects

Nurul Izzati Binti Azman

Plant Resource and Management Programme
Faculty of Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

Zingiberaceae is one of the family under the order Zingiberales. *Amomum* is one of the largest genus under family Zingiberaceae. Although *Amomum* is one of the largest genus, this genus is poorly known among ginger. The identification of this species is still confusing although the plant already been long utilized since there are a lot of species under genus *Amomum* and herbarium specimen are also incomplete. Thus, this project studied the morphological and anatomical characteristics of fruit from the selected *Amomum* species in Malaysia and use the data for species determination. The sampling areas were taken from various localities in Malaysia and 12 species of *Amomum* were examined. The method is the whole observation of the sample and all the morphology and anatomy of the fruit were observed. To ease this process, the Scanning Electron Microscope (SEM) and USB Digital Microscope were used. Most of *Amomum* fruit has the common morphology characteristics such as fruit shape in oval, capsule types of fruits with persistent calyx, presence of bracts and the arrangement in agglutinate.

Keywords: *Amomum*, Zingiberaceae, fruit, morphology, anatomy, Malaysia, systematic study, Scanning Electron Microscope (SEM).

ABSTRAK

Zingiberaceae adalah salah satu famili di bawah order Zingiberales. Amomum adalah salah satu genus terbesar di bawah keluarga Zingiberaceae. Walaupun Amomum adalah salah satu genus terbesar, genus ini kurang dikenali di kalangan halia. Pengenalpastian spesies ini masih mengelirukan walaupun tumbuhan ini telah lama digunakan kerana terdapat banyak spesies di bawah genus Amomum dan spesimen herbarium juga tidak lengkap. Oleh itu, projek ini telah mengkaji ciri-ciri morfologi dan anatomi buah daripada spesies Amomum terpilih di Malaysia dan menggunakan data tersebut untuk menentukan spesies. Kawasan persampelan telah diambil dari pelbagai tempat di Malaysia dan 12 spesis Amomum telah diperiksa. Kaedahnya adalah pemerhatian keseluruhan sampel dan semua morfologi dan anatomi buah telah diperhati. Untuk memudahkan proses ini, Mikroskop Imbasan Elektron (SEM) dan USB mikroskop digital telah digunapakai. Kebanyakan buah Amomum mempunyai karekter morfologi yang sama seperti bentuk buah yang bujur, jenis buah kapsul dengan kaliks yang kekal, kehadiran pelepah dan susunan buah yang melekat.

Kata kunci: *Amomum, Zingiberaceae, buah, morfologi, anatomi, Malaysia, kajian sistematik, Mikroskop Imbasan Elektron (SEM)*

CHAPTER 1

INTRODUCTION

Amomum Roxb. is one of the largest genus in the family Zingiberaceae which also known as ginger family. According to Kiew (1982) and Smith (1985), genus *Amomum* are widely distributed from Himalayas to Northern Australia which is in Southest Asia and until the central Pacific. Generally, species from *Amomum* genus are evergreen herbs occur in wet forests (Sakai & Nagamasu, 1998). Most of species from the genus *Amomum* commonly use as spices such as *Amomum capulaga*, vegetables, and condiment.

The identification of this species is still confusing although the plant already been long utilized since there are a lot of species under genus *Amomum* and the herbarium specimen are incomplete (Kaewsri & Paisooksantivatana, 2007). There are a lot of species available under *Amomum*, in order to compare all the species under genus *Amomum* properly, further study is needed to study to clarify the different between them and fruit are chosen as parameter in this study.

In this study, the parameters included were morphology, anatomy and micro-morphology that were observed using Scanning Electron Microscope (SEM) and also USB digital microscope in which made this study broader. The herbarium specimen is made and kept for the future referencing. After all data been recorded and analyse, it is expected that the species from genus *Amomum* are more easy to differentiate using the fruit since fruit is the most distinct features available in all plants.

The genus *Amomum* in Malaysia is poorly known among ginger and need more research and as well as the species which are difficult to describe because most of the herbarium specimen are incomplete. There are a lot of species available under *Amomum* genus, in order to compare all the species under genus *Amomum* genus properly, further study are needed to study to clarify the different between them and fruit are chosen as parameter in this study. Therefore, the objectives of this study are:

a) To study the morphological and anatomical characteristics of fruit of selected *Amomum* species from Malaysia.

b) To compare the morphological and anatomical characteristics of the fruit of selected *Amomum* species from Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Order Zingiberales

Order Zingiberales is morphologically diverse and a species-rich order that suited for particular studying of evolution of floral diversity. Banana, tumeric, ginger are some of the familiar plant that included in the order of Zingiberales. Under order Zingiberales, there are eight families which are Cannaceae, Costaceae, Heliconiaceae, Lowiaceae, Marantaceae, Musaceae, Strelitziaceae and Zingiberaceae. In the area before 1800s, the classification for the family under order Zingiberales are placed differently which is different from nowadays classification. The old version was that, Costaceae are placed under the same family as Zingiberaceae while Strelitziaceae, Lowiaceae, and Heliconiaceae is part of family Musaceae. Cannaceae and Marantaceae are already one of the families under order Zingiberales. Some phylogenist are managed to reconstructed the phylogenic history where they recognized the eight families under order Zingiberales, Cannariae and Zingiberariae as two superfamilies, and five suborders within the order. The attempt of reconstruction of the phylogenic history is done by several phylogenist which are Lane, Tomlimson, Dahlgren and Rasmussen (Kress, 1990). Since the day they have been found, this order showed a great evolutionary.

According to Kirchoff *et. al.* (2009), most members of the order has showy and large flower which demonstrated specialized relationship with pollinator. The stems in Zingiberales are commonly underground and short. The flower having three sepals, petals

carpel and its stamen up to six in two whorl of three each, and have three carpel. The most common stem in Zingiberales is short and below the ground. The branches arise from underground stem which are known as rhizome when it is elongated but a sucker when it is short. Most of the flowers has three sepals, three petals, up to six stamen in two whorl of three each and has three carpel.

The ovary is inferior which is enclosed by the united basal portion of other flower parts which later arise at the upper part of the ovary. Except for Zingiberaceae, the calyx is different from the corolla from the aspect of size and shape meanwhile the sepals are free from each other. Zingiberaceae has a tubular calyx with small free lobes. The flowers are zygomorphic or bilaterally symmetric but in certain cases they can be asymmetric too. Exhibit five stamen or one functional stamen in most flowers but not for all genus (Kirchoff *et al.*, 2009).

In the study of phylogeny of family under order Zingiberales, Sass *et al.,* (2016) conducted a study to capture sequences that relatively high in polymorphisms to detect the diversification that leading to the modern families. They used used transcriptomes that were generated as part of the Monocot Tree of Life Project (MonAToL) or One Thousand Plant Transcriptomes together with the whole genome of Musa acuminata recorded to design a set of probes that were printed on an Agilent microarray chip in parallel. This parallel printing approach enables divergent taxa to be captured on a single array and alleviates binding competition between closely related and divergent individuals. (Sass *et. al.,* 2016). Thus, finding on this study showed that, as shown in Figure 1, with 100% parsimony bootstrap, Musaceae is a sister to all other families and thus proves that the

gingers families (Cannaceae, Costaceae, Zingiberaceae and Marantaceae) are monophyletic (*Sass* et al., 2016).

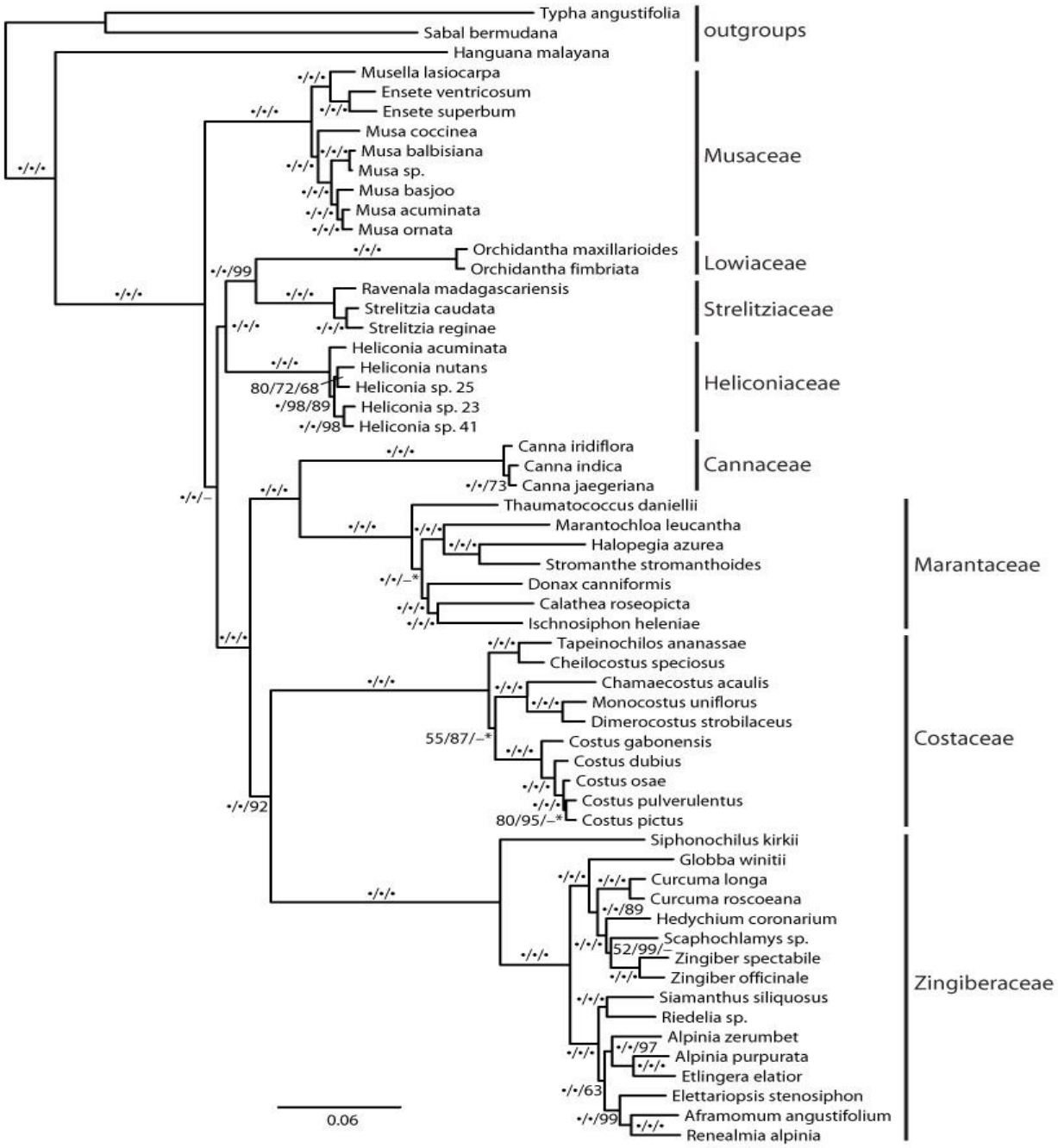

Figure 1: Phylogenetic tree of Zingiberales based on a partitioned ML of concatenated plastid and nuclear sequence.

2.2 Family Zingiberaceae

The members of family Zingiberaceae are rhizomatous, perennial herbs which are aromatic in any or all parts of the plant and it is comprises of 53 genera and more than 1500 species worldwide and its centre of diversity is at the Southeast Asia which has 80% of all known species (Lamb *et. al.*, 2013). It is the biggest family in the order Zingiberales which include some important spices such as *Zingiber officinale* Roscoe (ginger), *Curcuma longa* LINN. (turmeric), and *Elettaria cardamomum* L. (cardamom).

According to Smith (1985), species from the Zingiberaceae family usually are plants of the forest floor. It also diverse in its habits and habitat thriving in shaded forests, mixed dipterocarp kerangas, riverine habitat, secondary forest or alluvial forest with few species of montane and limestone areas. The majority of its species is also a terrestrial with some species occurs as epiphytes or litophytes (Ibrahim *et. al*, 2010). The characteristics of this family is that fusion of lateral staminodes of the inner staminal whorl into labellum, the occurrence of a cell that containing essential or ethereal oils that are automorphies to the family and also the presence of the two epigynous nectar glands at the base of the style of its flower (Kress, 1990).

Stated that there are 21 genera with over 200 species have been reported from Borneo (Poulsen, 2006) including *Haniffia* as a new generic record for Sarawak (Wong *et. al.*, 2014). Recent study according to Gobilik and Mashitah (2005), stated that Sarawak has 141 species in 18 genera, Brunei 110 species in 13 genera and Sabah 152 species in 13 genera which all include in Borneo. (Lamb *et. al.*, 2013)

2.3 Genus *Amomum* Roxb.

Amomum is one of the genus under family Zingiberaceae. They occur from the Himalaya to Northern Australia and extend into the central Pacific and widely distributed in Southest Asia (Kiew, 1982; Smith, 1985). According to Wu and Larsen (2000), most of spesis in the genus *Amomum* are widely creeping rhizomes, their leaf blade mostly oblong-lanceolate in shape, the inflorescene rising from the rhizomes, and lastly the fruit is often in capsule. According to Kaewsri and Paisooksantivatana (2007) in their study of *Amomum*, with 150-180 species, *Amomum* become one of the largest genera in the family Zingiberaceae.

Previous study by Schuman (1904), he published his monograph of the Zingiberaceae in "Das Pflanzenreich". He accepted Roxburgh's generic concept to remove Linnaean species from the genus and retained the name *Amomum*. He also subdivided *Amomum* genus into two sections and four series. Section *Geanthus* which distiguished by the absence of an anther appendage and composed of series *Oliganthae* and *Polyanthae*. Section *Euamomum* with an anther appendage, it comprised of series *Lobulatae* and *Integrae*. The four members of series *Oliganthae* have been subsequently transferred to the genus *Etlingera* Giseke and also most members of series *Polyanthae*, while including several true *Amomum*, may be more appropriately transferred to that genus as well. Majority of much larger section *Euamomum* are retained in genus *Amomum*. Members of family *Lobulatae* are distinguished by a bilobed or triloped anther connective and series *Integrae* by an entire anther connective. Besides, he purposed that 40 African *Amomum* species should be excluded and exstablished in genus *Aframomum.* This new genus was distinguished by the shape of fruit and the trilobed anther connective, whereas the

reformed genus *Amomum* was wholly Asiatic. Several species transferred to four other genera such as *Alpinia, Curcuma, Elettaria and Zingiber*.

According to Boyce (2006), currently there is no accurate number of *Amomum* in Sarawak and some suggest that the number is more than 50 species. Many species from the genus Amomum has been utilized as medicine, spice, condiment, and vegetable. The plant from this genus has been long utilized, however, the identification is still confusing since there are no good specimens in many herbaria and may lead to confusion and need more intensive study (Kaewsri & Paisooksantivatana, 2007). For instant, many species of Amomum were transferred to the other genera such *as Aframomum, Elettariopsis, Alpinia, Etlingera,* and *Hornstedtia.*

2.4 Fruit Morphology

Fruits are adaptation that results from the protection and the distribution of the seed. The fruits and seeds are mostly dispersed by animals but sometimes there are also another disperser such as wind, gravity and water. Fruits that are though and full of fibre or sclereids, such as walnuts, brazilnuts and coconuts have a maximum protection but they are heavy and somehow expensive metabolically. Furthermore, a fragile fruit is better for the germination to occur as if the fruits are hard to be broken, the seed cannot be easily germinate. If animals are going to disperse the seeds, part of the fruits must be edible or at least attractive to attract the animals while the seed must be protected from consumption.

Fusion of carpels also affects the nature of the fruit, if the fruit developed from a single ovary or the fused ovaries of one flower, it is a simple fruit. Meanwhile, if a separate carpel of one gymnoecium fuse during the development, aggregate fruits will form and if during the development all the individuals fruit of an inflorescences fuse into one fruit, it is a multiple fruit (Mauseth, 2012). In fruits, there are also a several layers such as epicarp, mesocarp, and endocarp which known as pericarp. Morphological study is the study of external part of the plant structure. According to Sattler and Rutishauser (1997), plant morphology are remains relevant to practically all disciplines of plant biology such as molecular genetics, physiology, ecology, evolutionary biology and also systematics. Study conducted by Kaewsri and Paisooksantivatana (2007) revealed the morphology of *Amomum* fruit from Thailand but the study are not in detail.

2.5 Fruit Anatomy

Anatomical study is the study of internal part of the fruit and can also be called as phytotomy. Anatomy study deals with the structural organization of the fruit. Since the middle 20th century, the investigations of plants anatomy considered as separate distinct field and plant anatomy refer to just internal plant structures. In study by Kaewsri and Paisooksantivatana (2007), the fruit anatomy is obtained, but there are no detail description on the anatomy of the fruit such as the placentation types and the colour of the pericarp being made. Schuman (1904) that purposed 40 African *Amomum* species should be excluded and exstablished in genus *Aframomum,* used shape of fruit to distinguished the new genus and the trilobed anther connective.

2.6 Scanning Electron Microscope (SEM)

Scanning Electron Microscope (SEM) can be used to view structure of the object that cannot barely seen by the naked eyes and also examine microscopic objects at a very fine scale. Instead of light using in the other usual microscope, SEM shot electron particles along with its high spatial resolution which will resulted in specific and informative image up to the scale of nanometre. SEM use electromagnets instead of lenses which result in much control in the degree of magnification up 15,000X. Previous study conducted by Kaewsri and Paisooksantivatana (2007) using the SEM as tool to view the pollen grains of *Amomum* in details. Clear image of the pollen are obtained. There is no study of fruit from genus *Amomum* using SEM found.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials and Sites

The samples were collected from various localities in Malaysia that include Kelantan (Gua Musang), Pahang (Cameron Highland), Sarawak (Kuching, Serian-Gunung Payang, Bau-Dered Krian, Miri-Long Banga) and also Universiti Malaysia Sarawak (UNIMAS). Twelve species of *Amomum* with fruits have been examined.

Figure 2: The Map of Malaysia (Source: Google Map)