COMPARISON STUDY ON MORPHOLOGY AND ANATOMY CHARACTERISTIC OF *PARACOSTUS BULLATUS* (MEEKIONG) AND *PARACOSTUS EBURNEUS* (MEEKIONG) FROM BAU, KUCHING DIVISION

Noor Ain Binti Che Ahmad

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Comparison Study on Morphology and Anatomy Characteristics of *Paracostus bullatus* (Meekiong) and *Paracostus eburneus* (Meekiong) from Bau, Kuching Division.

Noor Ain Binti Che Ahmad

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APPROVAL SHEET

Name of candidate : Noor Ain Binti Che Ahmad

Matric Number : 24330

Title of Project : Comparison Study on Morphology and Anatomy Characteristics of *Paracostus bullatus* (Meekiong) and *Paracostus eburneus* (Meekiong) from Bau, Kuching Division.

(Prof. Dr. Cheksum @ Supiah Tawan)

Supervisor

(En.Qammil Muzzammil bin Abdullah)

Co-supervisor

(Dr. Siti Rubiah Zainudin)

Coordinator

Plant Resource Science and Management Programme

Department of Plant Science and Environmental Ecology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

DECLARATION

The work presented in this final year project report is the result of my own research except as cited in the references. No portion of the work refer to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institutions of higher learning.

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Noor Ain binti Che Ahmad (24330)

Plant Resource Science and Management Programme

Department of Plant Science and Environmental Ecology

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

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ABBREVIATIONS

HUMS	=	Herbarium Universiti Malaysia Sarawak
UNIMAS	=	Universiti Malaysia Sarawak
FRST	=	Faculty Resource Science and Technology
SEM	=	Scanning Electron Microscope
CO_2	=	Carbon dioxide
$CaCO_3$	=	Calcium carbonate
$CaMg(CO_3)$	=	Dolomite
SiO_2	=	Silica dioxide
Na_2HPO_4	=	Disodium phosphate
$Na_2HPO_4.H_2O$	=	Dihydrogen sodium phosphate

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Noor Ain Binti Che Ahmad

Plant Resource Science and Management
Department of Plant Science and Environmental Ecology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak

ABSTRACT

The Order Zingiberales is a monocotyledonous plant that consists of eight families with 92 genera and approximately 2111 species. The Costaceae is one of the Zingiberales members that famously known as spiral gingers or locally known as Setawar hutan due to it having spiromonostichous leave arrangement. This family recently has been revised and comprises seven genera, namely *Costus*, *Chamaecostus*, *Cheilocostus*, *Dimerocostus*, *Monocostus*, *Paracostus* and *Tapeinochilos*. The genus *Paracostus*, previously represented by only two species now has increased to six species. Among the species, *P. bullatus* (Meekiong) and *P. eburneus* (Meekiong) were the two hyper endemic species that found from Bau Limestone areas. These two species shared several characteristics and only distinguished by the floral characteristics. Therefore this project aimed to investigate details on differences on the aforementioned species via morphological and anatomical aspects. Fresh samples were collected from the type localities. The morphological and anatomical characteristic of both species were determined. Observation and measurement of fresh collections were conducted to determine morphological characteristic of both species. The result showed both species clearly differed in term of leaves colour and floral characteristic. Leaf anatomical characteristic of both species were examined by using scanning electron microscope (SEM). The types of stomata on the adaxial leaf surface of both species were similar; paracytic, hemiparacytic and anomocytic but on abaxial leaf surface the types of stomata were differed; *P. bullatus* had paracytic, hemiparacytic and anomocytic while *P. eburneus* only had anomocytic. The longest average sizes of stomata on adaxial and abaxial leaf surface belong to *P. bullatus*. The longest average sizes of guard cells on adaxial leaf surface belong to *P. eburneus* while on the abaxial leaf surface belong to *P. bullatus*. Trichomes were presence only on abaxial leaf surface of both species; *P. bullatus* and *P. eburneus*. Both *Paracostus* species clearly differed in morphological and anatomical characteristic.

Keywords: *Paracostus*, *Costus*, Costaceae, Scanning electron microscopy (SEM), Sarawak

ABSTRAK

Order Zingiberales merupakan tumbuhan monokot yang terdiri daripada lapan famili dengan 92 genera dan kira-kira 2111 spesies. Costaceae merupakan salah satu famili dalam Zingiberales yang dikenali sebagai Halia hutan atau Setawar hutan kerana ia mempunyai susunan daun spiromonostichous. Baru-baru ini, famili ini telah dikaji dan dibahagikan kepada tujuh genera, iaitu *Costus*, *Chamaecostus, Cheilocostus, Dimerocostus, Monocostus, Paracostus* dan *Tapeinochilos*. Sebelum ini, genus *Paracostus* hanya diwakili oleh dua spesies tetapi sekarang telah meningkat kepada enam spesies. Antara spesies tersebut, *P. bullatus* (Meekiong) dan *P.eburneus* (Meekiong) merupakan dua spesies hiper endemik yang ditemui di kawasan Batu Kapur, Bau. Kedua-dua spesies berkongsi beberapa ciri yang sama dan hanya dapat dibezakan melalui ciri-ciri bunga. Oleh itu, projek ini bertujuan untuk mengkaji dengan lebih terperinci perbezaan yang telah dinyatakan dari aspek morfologi dan anatomi. Sampel segar dikumpulkan daripada tip lokaliti. Ciri-ciri morfologi dan anatomi kedua-dua spesies telah ditentukan. Pemerhatian dan pengukuran koleksi segar telah dijalankan untuk menentukan ciri-ciri morfologi bagi kedua-dua spesies. Keputusan menunjukkan kedua-dua spesies mempunyai perbezaan dari segi warna daun dan ciri-ciri bunga. Ciri-ciri anatomi daun untuk kedua-dua spesies telah diperiksa dengan menggunakan pengimbas electron mikroskop (SEM). Jenis stomata di bahagian permukaan atas daun bagi kedua-dua spesies adalah sama; paracytic, hemiparacytic dan anomocytic tetapi pada bahagian bawah permukaan daun, jenis stomata adalah berbeza; *P. bullatus* mempunyai paracytic, hemiparacytic dan anomocytic manakala *P. eburneus* hanya mempunyai satu jenis stomata; anomocytic. Purata saiz stomata yang paling tinggi di bahagian atas dan bawah permukaan ialah *P. bullatus*. Purata saiz sel pengawal bagi bahagian atas permukaan daun ialah *P. eburneus* manakala bagi bahagian bawah permukaan ialah *P. bullatus*. Kedua-dua spesies *Paracostus* jelas berbeza dari segi ciri-ciri morfologi serta ciri-ciri anatomi daun.

Kata kunci: *Paracostus, Costus*, Costaceae, pengimbas electron mikroskop (SEM)

CHAPTER 1

INTRODUCTION

Borneo is the third largest island in the world which represents one of the most biologically diverse collection planets of ecosystems (MacKinnon, 1997). It covers an area of approximately 745,567 square kilometers. The island consists of three countries which are Malaysia including Sarawak and Sabah, Indonesia (Kalimantan) and Brunei which is encompassed by Sarawak. It is located on the equator and has a rainy humid equatorial climate that receives approximately 4,000 to 5,000 millimeter of rain every year (Pio & Dorethea, 2005). There are high mountains which provide many different habitats. Borneo is considered to be a center of biodiversity in tropical Asia based to the wide variety of plant species that developed earlier as a result of its isolation from the continent (Hazebroek & Morshidi, 2001).

There are a few types of forest in Borneo. The main four type forest is lowland rainforest, montane forest, inland swamp forest and mangrove forest. More forest was disappeared due to the development of shifting agriculture, commercial logging and clearing land for large scale agriculture. There are 64 families, 224 genera and 1201 species represent only a quarter of the Bornean tree flora. About 490 species was identified as Bornean endemics and about 102 species was identified endemic to Sarawak. The total of flora that endemic in Sarawak may eventually reach 400 species (Soepadmo, 1999).

Limestone is a sedimentary rock that consists of the mineral calcite (calcium carbonate, $CaCO_3$). The other common mineral in limestone is dolomite [CaMg (CO_3):] while the other common impurities in limestone include chert (microcrystalline, cryptocrystalline quartz or amorphous silica, SiO_2), clay, organic matter and iron oxides (Chan, 1986). Limestone formations are found throughout Borneo and include extensive areas in the Peninsula and the limestone hills of Sarawak. The distribution of limestone formation is influenced by the geological evolution of the region. Detailed botanical surveys of the limestone areas have not been completed, but preliminary studies suggest that this habitat supports a tremendous number of flora species where many of them probably endemic (Whitmore, 1989).

According to Bandarin (2001) limestone area provide high levels of radiation and endemism in both plant and animal species which including the little studied subterranean fauna of caves. There are wide ranges compositions of flora and fauna presents in the limestone area. Fauna that presents in the limestone area consist of both vertebrates and invertebrates. The number of species of trees that was estimated in Borneo is about 5, 000 of which about 41% are expected to be endemic to the island. There are only seven out of the 2000 odd species that make up the limestone flora in Malaysia show any adaptation for dealing with excess calcium ions (Burtt & Tan, 1984).

Bau limestone area can be geographically defined including the whole parts of limestone formation in the southern portion of Kuching Division which located in the Bau-Serian, west Sarawak (Liechti *et al.*, 1960). The thickness of the limestone was estimated about 300 m in the Jagoi area and 500 m just south of Bau Town.

The inventory of Bau limestone area has reported species which endemic to Bau comprises of *Begonia congesta, Monophyllaea glauca var boraginea, Monophyllaea singularis var singularis* and *Nepenthes northiana*. This study showed that the flora and fauna of the Bau limestone area is rich and distinctive with many of species rare and endemic.

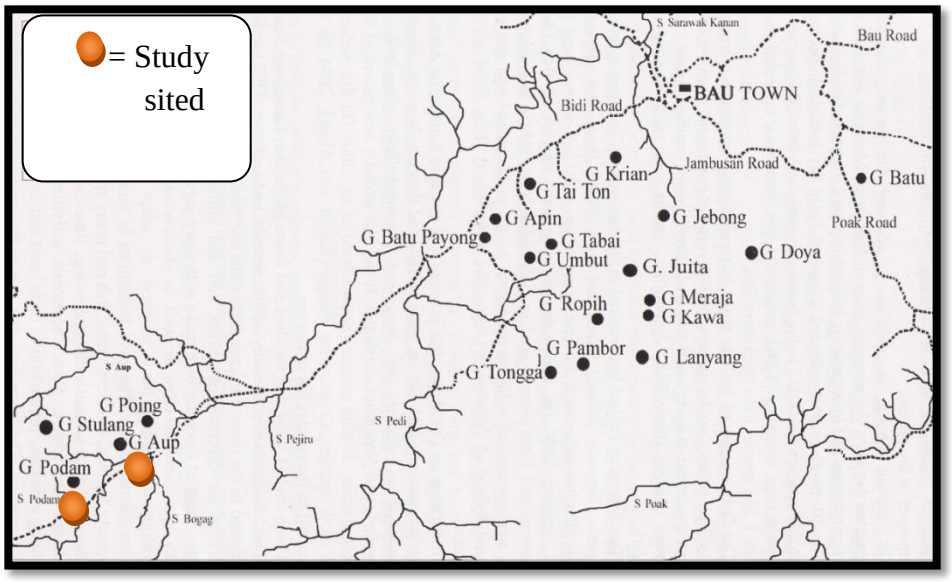


Figure 1: Bau Limestone area

Tropical gingers (Zingiberales) belong to the order of the monocotyledonous plants (Liliopsida). The order has eight families with 92 genera and about 2111 species. The families have been divided into two groups based on functional stamen (Larsen *et al.*, 1999). They are distinctive with many unique and highly modified morphological characteristics (Kirchoff, 1991). The spiral gingers family Costaceae has previously been treated under Zingiberaceae (Maas, 1979).

The genus *Costus* is the largest genus in Costaceae and majority are pantropical with its greatest diversity centered in the neotropics (ca. 40 spp.); 25 species occur in tropical Africa and only 5 species in southeastern Asia. Recent study by Meekiong *et al*., (2006) added four new described species into the Asian's List.

Two recently described species *P. bullatus* (Meekiong) and *P. eburneus* (Meekiong) are considered hyper endemic to Bau limestone areas that only found from a single limestone hill each. Taxonomically both species are sharing similar characteristics in terms of appearances such as bullate leaves and calcicolous habitat but only differs by the floral morphological. Therefore, the aims of this study are:-

a) to compare the morphological structure of *P. bullatus* and *P. eburneus.*
b) to study the leaf anatomical structure of *P. bullatus* and *P. eburneus* by using scanning electron microscope (SEM).
c) to produce the details morphological account of both *Paracostus* species.

CHAPTER 2

LITERATURE REVIEW

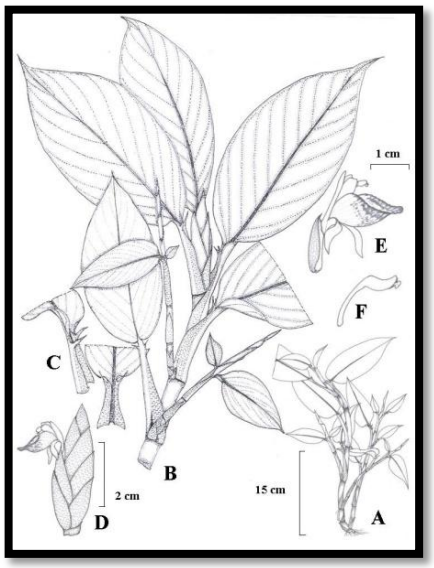
2.1 History Order of Zingiberales

According to Tomlinson (1962) the Zingiberales is a very natural order of monocotyledons whose members are almost entirely restricted to tropical regions. Taxonomist was recognized the order as a natural entity because of the comparable in its homogeneity to grasses, palms or orchids. There are eight families in this order which can be found mainly in the tropical and subtropical regions.

Dahlgren & Clifford (1982) formed two informal groups are often recognized based upon shared morphological and anatomical characters. There are the paraphyletic basal group which consist of Musaceae, Heliconiaceae, Strelitziaceae, Lowiaceae and the other one is the monophyletic group which comprise of Zingiberaceae, Costaceae, Cannaceae, Marantaceae. The family are divided into two informal group based on functional stamens. The group with five functional stamens are consists of Musaceae, Lowiaceae, Heliconiaceae and Strelitziaceae. While, the other group which known as more advance families with one functional stamen are Cannaceae, Costaceae, Marantaceae and Zingeberaceae (Larsen *et al*., 1999).

The morphological and anatomical characters that differentiate the order were described by Tomlinson (1962). The morphological included the rhizomatous herbs have leaf with open where sometimes ligulate sheaths; lamina entire with lateral veins diverging from a common midrib and one-half of blade completely rolled around the other during development.

Based on Zingiberales evolution, several significant are changes in floral morphology involving the perianth and androecial whorls (Kress, 1990; Rudall & Bateman, 2004). Flowers in the Zingiberales have two trimerous whorls of tepals, two trimerous androecial whorls and a tricarpellate gynoecium. Flower is zygomorphic, perianth consisting of separate calyx and corolla. Fertile stamens usually five or one and one to five stamens usually represented by staminodes. All of the family in this order is identify at some level of the taxonomic hierarchy. Based on the morphological analyses done by Stevenson & Loconte (1995) and Chase *et al.*, (1995), this order was formed a clade.



A: Rhizomatous herb

B: Young leaf roll and present or midrib

C: Present of ligule

D: Involucral bract

E: Stamen and filament formed staminode structure

F: Zigomorphic flower

Figure 2: Unique characteristic of Zingiberales

2.2 Description of Family Costaceae

Costaceae is a pantropical family of monocots consisting of approximately 120 species which is easily recognizable groups within the order Zingiberales. The studied by Petersen (1889) and Schumann (1904) cited in Kress *et al*., (2002) showed that Costaceae was previously included in the family Zingiberaceae because of was largely based on broad similarities of inflorescence and floral character. However, recently Specht *et al.,* (2001) segregated Costaceae as it own family in the order of Zingiberales because of a number of distinctive characters such as lack of aromatic oils, branched aerial stems and spiral monostichous (one sided) phyllotaxy is difference compared to the Zingiberaceae.

Kirchoff (1988) explained that the Costaceae has a unique floral structure which is only develops a single fertile stamen while the remaining of five infertile stamens will fused together to form a large petaloid labellum that dominates the floral display. Schumann (1904) reported that, the genus *Costus* was divided into five subgenera; viz. *Costus*, *Epicostus*, *Metacostus*, *Paracostus*, and *Cadalvena* based upon general characteristics of overall floral morphology (Meekiong *et al.*, 2006).

Previously, only four genera were recognized as by Larsen *et al.*, (1999) which were *Costus, Dimerocostus, Monocostus* and *Tapeinochilos*. However, Specht (2006) proposed reclassification of Costaceae was based on molecular phylogeny of available taxa studied. The genus *Costus* was divided into four genera; *Costus*, *Chamaecostus, Cheilocostus* and *Paracostus* where the last two was a segregation of the Asian's *Costus*.

She suggest that all of the *Costus* species from Asia apart from *C. paradoxus* be transferred to the genus *Cheilocostus*, whereas the Bornean *C. paradoxus* and the African species, *C. englerianus* were placed under the genus *Paracostus*. There are only two genera, *Cheilocostus* and *Paracostus* that are native to Borneo with seven species of Costaceae are presently known (Maas, 1979; Meekiong *et al.,* 2006, 2008).

2.3 Ecology and Distribution of *Paracostus* Species at Sarawak

According to Meekiong *et al.,* (2006) the genus *Costus* was widely spread in Sarawak. The ecology of *Cheilocostus* and *Paracostus* species are difference. The genus *Cheilocostus* usually was found in damp and wet places area like near small streams or rivers. For example, the commonest species which is *C. speciosus* was found from lowland peat swamp areas to more than 800 m. altitude. This species had been known as epiphytes on the tree trunks. While, *Paracostus* species usually found growing in shady, wet or damp areas of limestone foothills, Mixed Dipterocarp and Kerangas forest (Refer Appendix 1).

The members of genus *Paracostus* are usually found in moderate to big population patches which is rarely in solitary clumps like other members of *Cheilocostus*. This species looks more successful in producing suckers and will form the big clumps. They are rarely growth from the seedlings and usually produce the small fruit. The study showed that, the pollinators and dispersal agents for this species are presently unknown. Based on our field observations and Meekiong *et al.,* (2006) discovered that the seeds were dispersed washed away by heavy rainfall.

Besides that, there were species which known as *Monophyllaea glauc*a; a species that was unique and thrived well on the limestone area. *Monophyllaea glauc*a was used in medicinal remedy by the local people. This species growing predominantly on limestone rocks, in shady forests at cave entrances and below rocks at limestone area (Refer to Appendix 1).

Other *Paracostus* species known as *P. paradoxus* was recorded from Belaga, Matang and Sabal, Sarawak. This species was found at hill mixed dipterocarp forest which growing in semi shady areas, damp and wet places near a small stream. It had been recorded flowering on February, August and November. Another species was *P. muluensis* that was recorded from Belaga and Miri, Sarawak. It grows well in Mixed Dipterocarp and Kerangas forest. It also grows in clump formed in damp and wet places near to the streams or rivers. The flowering was recorded from November to April. This species had long time been misidentified as *P. paradoxus*. It was easily recognized by the white labellum with fan-shaped yellow patch on the throat. But, it was differed compared to *P. bullatus* and *P. eburneus* (Meekiong *et al.*, 2006). (Refer to Appendix 2).

2.4 Scanning Electron Microscope (SEM)

Based on Troughton & Donaldson (1972), scanning electron microscope (SEM) was made with reference to some anatomical features in plants and discussed the relationship of these structures to physiological processes. It involves micrographs of the leaf surface, stomata, inside the leaf, chloroplast, xylem, the apical meristem, flowers and seed development. SEM is one of a powerful method was used to study the surface structures of mollicutes. This technique provides a large depth of field where the area of the sample that can be viewed in focus at the same time is actually quite large (Flegler *et al.*, (1993).

According to Balbi *et al.*, (2009) SEM is a useful tool for the morphological study of different organs and structures. SEM is one of the significant tools that being used in the Plant Systematic study. SEM is scientific instruments that use a beam of energetic electrons to examine objects on a very fine scale. This required 200-1000 x plus magnification which are not possible using current optical microscopes. The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time and produces an image that is a good representation of the three-dimensional sample. The combination of higher magnification, larger depth of field, greater resolution, compositional and crystallographic information makes the SEM is one of the most heavily used instruments in academic, national lab research areas and industry. SEM enables details study on miniature anatomical structures that cannot be seen using bare ayes. SEM will be used in this study to get the clear image of differences of the leaves of *P. bullatus* and *P. eburneus* (Anon, 2011).

2.5 Stomata

Stomata are small pores which usually on the undersides of leaves that are opened or closed under the control of a pair of banana-shaped cells called as guard cells. Stomata are assumed to be characteristic for the aerial generation. The function of stomata is important on the physiology, adaptation and productivity of plants. According to Esau (1977) stomata are found on most aerial surface of plants including the adaxial epidermis, stems and flowers. Some of the species have stomata on abaxial and adaxial surfaces of leaves. Different species have different structure of stomata. Stomatal structures vary greatly in concern with species habitat and leaf architecture. There are large heritable differences between species in stomatal dimensions, distribution and morphology. Jones & Sutherland (1991) have proposed that the main role of stomata perhaps to avoid damaging of plant water deficits.