KAJIAN SISTEMATIK KE ATAS GENUS *PLAGIOSTACHYS* RIDL. (ALPINIOIDEAE: ZINGIBERACEAE) DI BORNEO

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DECLARATION

The materials in this thesis are original except for quotations, excerpts, summaries and references, which have been duly acknowledged.

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ABSTRACT

The genus *Plagiostachys* of Borneo was revised. Morphological characteristics in living plants were observed to find new significant characters for the genus classification. This study showed that several characters, such as the shape and the presence or absence of anther crest, the branching pattern, and the bracteole shape were consistent within a species and taxonomically important to differentiate between the closely related species. Seven new species and one new variety were recognized, described and illustrated, namely *P. brevicucullata* A. Julius & A. Takano, *P. longicaudata* A. Julius & A. Takano, *P. megacarpa* A. Julius & A. Takano, *P. pustulosa* A. Julius & A. Takano, *P. roseiflora* A. Julius & A. Takano, *P. subulata* A. Julius & A. Takano, *P. viridisepala* A. Julius & A. Takano and *P. breviramosa* J. Cowley var. *ecristata* A. Julius & A. Takano. A key to species of Bornean *Plagiostachys* was constructed based on morphological characteristics. Molecular phylogenetic analysis using nuclear DNA sequence data of internal transcribed spacer region (ITS1, ITS2) and 5.8S gene was conducted to examine the phylogenetic status of the genus. The strict consensus tree (Length = 1216; CI = 0.4120; RI = 0.7395; RC = 0.3047) obtained from parsimony analysis of the ITS sequence data showed that the species of *Plagiostachys* made a strongly supported clade with some *Alpinia* species which belong to section *Alpinia* (BS = 92 %). On the other hand, species of *Plagiostachys* comprise three subclades and each subclade is moderately to strongly support with relatively high bootstrap value: *Plagiostachys* subclade A (BS = 70%), *Plagiostachys* subclade B (BS = 81%) and *Plagiostachys* subclade C (BS = 99%). *Alpinia* is not included into the subclades. It suggests that the *Plagiostachys* is a different entity from *Alpinia* but more data is needed to verify this. Therefore, *Plagiostachys* remains in its current status until further analysis is carried out. The ITS sequences also provided new data for inferring relationships within *Plagiostachys* and allow new interpretations of capsule character (pubescence or glabrous capsule) that maybe of value in future classification.

ABSTRAK

KAJIAN SISTEMATIK KE ATAS GENUS PLAGIOSTACHYS *RIDL. (ALPINIOIDEAE: ZINGIBERACEAE) DI BORNEO*

Kajian telah dibuat ke atas genus Plagiostachys *di Borneo. Ciri-ciri morfologi ke atas tumbuhan hidup telah dikaji untuk mencari ciri-ciri penting bagi pengkelasan genus tersebut. Hasil kajian ini menunjukkan bahawa beberapa ciri seperti bentuk dan ada atau tidak ada 'anther crest', corak percabangan dan bentuk 'bracteole' adalah konsisten di dalam spesies dan penting secara taksonomi untuk membezakan di antara spesies-spesies terdekat. Tujuh spesies baru dan satu variasi baru telah dikenalpasti, dihurai dan diilustrasi, iaitu* P. brevicucullata *A. Julius & A. Takano,* P. longicaudata *A. Julius & A. Takano,* P. megacarpa *A. Julius & A. Takano,* P. pustulosa *A. Julius & A. Takano,* P. roseiflora *A. Julius & A. Takano,* P. subulata *A. Julius & A. Takano,* P. viridisepala *A. Julius & A. Takano dan* P. breviramosa *J. Cowley var.* ecristata *A. Julius & A. Takano. Kekunci spesies* Plagiostachys *di Borneo telah dibuat berdasarkan ciri-ciri morfologi. Analisis molekul filogenetik menggunakan data jujukan nuklear DNA, 'internal transcribed spacer region' (ITS1, ITS2) dan '5.8S' gen telah dibuat untuk mengkaji status filogenetik genus tersebut. Pokok 'strict consensus' (Panjang = 1216; indeks kestabilan = 0.4120; indeks penahanan = 0.7395; indeks terskala = 0.3047) yang diperolehi daripada analisis parsimoni data jujukan ITS menunjukkan bahawa gabungan spesies* Plagiostachys *dengan beberapa spesies* Alpinia *adalah disokong kuat (BS = 92 %). Sebaliknya, spesies* Plagiostachys *yang terdiri daripada tiga 'subclades' dan setiap 'subclade' disokong secara sederhana dan kuat mempunyai nilai 'bootstrap' yang tinggi secara relatif:* Plagiostachys *subclade A (BS = 70%),* Plagiostachys *subclade B (BS = 81%) dan* Plagiostachys *subclade C (BS = 99%). Spesies* Alpinia *tidak terdapat di dalam setiap 'subclade'. Ini menunjukkan bahawa* Plagiostachys *mungkin entiti lain daripada* Alpinia *tetapi data lanjut adalah perlu untuk mengesahkannya. Dengan itu, status semasa* Plagiostachys *dikekalkan sehingga kajian lanjut dilakukan. Penjujukan ITS juga memberikan informasi baru mengenai hubungan di dalam* Plagiostachys *seperti kapsul berbulu atau licin yang mana ciri tersebut adalah penting untuk pengkelasan di masa akan datang.*

KEYWORDS

Borneo, ITS, *Plagiostachys*, Systematic, Zingiberaceae

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LIST OF ABBREVIATIONS

BO	:Herbarium Bogor
BORH	:BORNEENSIS herbarium
BS	:Bootstrap value
CI	:Consistency Index
CRP	:Crocker Range Park
DVCA	:Danum Valley Conservation Area
E	:Herbarium Royal Botanic Gardens, Edinburgh
ETS	:External Transcribed Spacer
FI	:Herbarium Universitatis Florentinae
G	:Herbarium Conservatoire et Jardin botaniques de la Ville de Geneva
HI	:Homoplasy Index
HYO	:Herbarium of Museum of Human Nature & Activities, Hyogo
IBSC	:Herbarium South China Botanical Garden
ITS	:Internal Transcribed Spacer
JSPS	:Japan Society for Promotion of Science (BioTropical resources)
K	:Herbarium Royal Botanic Gardens, Kew
MBCA	:Maliau Basin Conservation Area
ML	:Maximum Likelihood
MP	:Maximum Parsimony
MULTREES	:Multiple trees
NHM	:Herbarium University of Nottingham
NNI	:Nearest-Neighbour Interchange
PCR	:Polymerase Chain Reaction
PR	:Herbarium National Museum in Prague
RC	:Rescaled Consistency Index
RDC	:Rainforest Discovery Centre
RI	:Retention Index
SAN	:Herbarium Sandakan
SAR	:Herbarium Sarawak
SING	:Herbarium Royal Botanic Garden, Singapore
SNP	:Sabah Parks herbarium
TBR	:Tree Bisection-Reconnections
matK	:*maturase*-K
rDNA	:ribosomal Deoxyribonucleic acid
rRNA	:ribosomal Ribonucleic acid
RDC	:Rainforest Discovery Center

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CHAPTER 1

INTRODUCTION

Zingiberaceae, commonly known as the ginger family, is the largest of the eight families comprising the monophyletic tropical order Zingiberales. The family form a monophyletic group together with Costaceae, Marantaceae, and Cannaceae, and Costaceae is the sister family to Zingiberaceae (Clark *et al.*, 1993; Kress, 1990). It is distributed pantropically with the center of diversity in South and South East Asia. 53 genera and over 1300 species are known in this family (Kress *et al.*, 2002). Of these, 24 genera and about 600 species occur in the Malesian region, throughout Malaysia, Indonesia, Brunei, Singapore, the Philippines, and Papua New Guinea (Larsen *et al.*, 1999). While for Borneo, there are 18 genera and over 200 species have been reported (Poulsen, 2006). Among these, the genera such as *Alpinia, Amomum, Boesenbergia, Etlingera, Hornstedtia* and *Plagiostachys* are commonly found in Borneo. Within the family Zingiberaceae, four tribes are traditionally recognized, namely Alpinieae Meisn., Globbeae Meisn., Hedychieae Petersen and Zingibereae Burtt & Olatunji (Burtt & Smith, 1972; Smith, 1982). However, the current classification based on DNA sequence data presented by Kress *et al.* (2002) has recognized four subfamilies; the Siphonochiloideae

W. J. Kress (the genus *Siphonochilus* only), the Tamijioideae W. J. Kress (*Tamijia*), the Zingiberoideae Haask. (including the former tribes Hedychieae and Globbeae), and the Alpinioideae Link (comprises most of the former Alpinieae). The subfamily Alpinioideae, which is characterized by the plane of distichy of leaves perpendicular to rhizome and the reduction or absence of the two lateral staminodes, are further subdivided into two tribes, i.e. Alpinieae A. Rich. and Riedelieae W. J. Kress. The Alpinieae, defined by having fleshy or indehiscent fruit and lacking extrafloral nectarines, includes all remaining former Alpinieae, e.g. *Alpinia, Amomum, Etlingera, Hornstedtia* and *Plagiostachys*.

Plagiostachys Ridl., is a complex genus and comprising about 24 species. It is distributed in Indochina to Malesia with the center of diversity in Borneo (Smith, 1985). The genus is generally the constituent of the undergrowth in the tropical forests. The plants inhabiting damp places in the primary forest, but sometimes they are found in old secondary forest and disturbed sites. It has been distinguished from the other genera in Zingiberaceae by the apparently lateral, tightly congested inflorescence, which is, in fact, terminal on the short stem of the leafy shoots, and breaks through the leaf sheaths usually just above ground level or sometimes in the middle (Smith, 1990). The flower is subtended by a usually tubular bracteole, and the labellum, although small and rather fleshy, is petaloid with divergent venation (for some species). The

inflorescence is, in certain species, very mucilaginous, where the bracteole and calyx are fleshy, and tend to decay soon.

In many instances, the taxonomic study of *Plagiostachys* have been devoted to Borneo (Gobilik *et al.*, 2005; Sakai & Nagamasu, 2003) compared to the other areas such as the Philippines, Indonesia and Peninsular Malaysia, as very few literature is available. The genus is still poorly known among gingers and needs critical revision and more research work (Larsen *et al.*, 1999; Smith, 1982).The problem is mainly because of the mucilaginous nature of the inflorescences. This hinders close examination of inflorescences and flowers in dried herbarium specimens. It is not only because of the flower structure will be lost but also the fleshy and decaying calyx and bracteole, virtually unusable when dealing with dry specimen. Another problem is, most of the herbarium specimens are incomplete and seem to have been collected in fruits rather than in flower (Cowley, 1999). Thus, studying on many living plants and doing observations in the field are the most important steps in revising the genus. These will enable one to gather many data on variation of important characters in the species of *Plagiostachys* that are useful for its classification.

The existing classification of Bornean *Plagiostachys* has been based on Smith (1985). She classified the genus into two informal groups: the Group I, defined by having mucilaginous inflorescences, the bracteoles distinctly tubular, early decaying

and only the very basal part remaining, the calyx is fleshy and decaying in the upper part at least, the style sometimes adnate to the wall of the corolla tube above the epigynous glands, the capsule is oblong-pyriform, angled and rarely globose, the ligule is bilobed, and the flowers are yellow and white with some pink; whereas the Group II is characterized by having non-mucilaginous inflorescences, the bracteoles tubular at base or open, and partially decaying or persistent, the calyx is not fleshy and not decaying, the style usually free from the wall of the corolla tube at the base, the capsule is globose, the ligule is truncate or emarginate and rarely bilobed, and the flowers is pinkish-red with a yellow labellum. The classification is, however, disputed by Cowley (1999) and Sakai & Nagamasu (1998) as the characters used for the grouping did not fit with any species described recently. This indicates Smith's grouping can no longer be used. Further investigation of the usefulness of the grouping in the present day is needed.

The study by Kress *et al.* (2005) is the most thorough paper to date addressing the relationships among genera in the subfamily Alpinioideae. The result of their molecular assessment shows that *Amomum* and *Alpinia* are polyphyletic groups and *Plagiostachys* embedded within *Alpinia*. *Alpinia* is the largest and most widespread genus in Zingiberaceae with more than 200 species (Larsen, 1998), occurring from India and Sri Lanka to China, Japan, Southeast Asia, New Guinea, Australia, Fiji and

Samoa (Smith, 1990). The genus, defined by having terminal inflorescences on the leafy shoots and the lateral staminodes are absent or represented by small tooth-like structures at the base of the labellum. This polyphyletic genus was splitted into six clades of species each with varying statistical support. The analysis of their combined data sets (ITS and *mat*K) have demonstrated that Clades I, II, III and IV are each strongly supported as monophyletic (BS > 90%), whereas Clades V (BS = 61%) and Clade VI (BS < 50%) are weakly to poorly supported groups. On the other hand, *Plagiostachys* is moderately to strongly supported (BS > 70%) as a monophyletic group, but they only included two species in their analyses. Therefore, the monophyletic status of *Plagiostachys* requires further confirmation by adding more species of the genus. It is also expected that by increasing the taxon sampling in the phylogenetic analysis will illustrate the relationships within *Plagiostachys* in order to see how congruent are Smith's (1985) informal grouping of the genus. Additionally, any attempt to reclassify *Plagiostachys* are should make reference to its closely related genus *Alpinia*. This is not only because *Alpinia* is the sister genus of *Plagiostachys* (Kress *et al.*, 2005; Pedersen, 2004), but the laterally produced inflorescence in the latter is also shared with *A. hansenii* R. M. Sm. and *A. havilandii* K. Schumn. (Smith, 1990). Both of these species are also apparently having inflorescence breaking through the leaf sheaths near the top and are, as far as is known, never mucilaginous.

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