BIOGEOGRAPHICAL INVESTIGATIONS ON THE SCHISMATOGLOTTIS NERVOSA COMPLEX (ARACEAE: SCHISMATOGLOTTIDEAE) IN BORNEO

April Ting Pei Jen

Bachelor of Science with Honours
(Plant Resource Science and Management)
2010
BIOGEOGRAPHICAL INVESTIGATIONS ON THE SCHISMATOGLOTTIS NERVOSA COMPLEX (ARACEAE: SCHISMATOGLOTTIDEAE) IN BORNEO

APRIL TING PEI JEN

This project is submitted in partial fulfilment of the requirements for the Degree of Bachelor Science with Honours in Plant Resource Science and Management

Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2010
APPROVAL SHEET

Name of candidate: April Ting Pei Jen

Title of dissertation: Biogeographical Investigations on the Schismatoglottis nervosa Complex

(Araceae: Schismatoglottideae) in Borneo

Dr. Wong Sin Yeng
Supervisor

Assoc. Prof. Dr. Ismail Jusoh
Coordinator of Plant Resource Science and Management Programme
Department of Plant Science and Environmental Ecology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
DECLARATION

I declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

April Ting Pei Jen
Plant Resource Science and Management
Department of Plant Science and Environmental Ecology
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
ACKNOWLEDGEMENT

A special thanks to Dr. Wong Sin Yeng and Peter Boyce for their guidance and support for the completion of the project. Appreciation also goes to master student, Low Shook Ling and fellow colleague, Low Shook Eng for their assistance and encouragement in times of difficulties. Thanks to the lab assistant, Madam Fatimah for her helps in providing chemicals and laboratory equipments. Lastly, with God’s blessings, I would like to express my gratitude to my family for their love and financial support throughout the studies in UNIMAS.
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LIST OF ABBREVIATIONS

MatK  Maturase K
DNA  Deoxyribonucleic acid
PAUP  Phylogenetic Analysis using Parsimony
HGT  Horizontal gene transfer
bp  Base pair
RNA  Ribonucleic acid
CTAB  Cetyl Trimethyl Ammonium
PVP  Bromide PolyVinyl Pyrrolidone
°C  Degree Celsius
NaCl  Sodium chloride
M  mole
µL  microlitre
%  percent
PCR  Polymerase Chain Reaction
MgCl₂  Magnesium chloride
dNTPs  Deoxynucleotide triphosphates
Taq DNA polymerase  *Thermus aquaticus* DNA polymerase
DEPC  Diethylpyrocarbonate
TBR  Tree-bisection-reconnection
AIC  Akaike Information Criterion
CI  Consistency index
RI  Retention index
RC  Rescaled consistency index
HI  Homoplasy index
ITS  Internal Transcribed Spacer
mya  Million years ago
TrnL-f
TrnK
PaupUp
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Biogeographical Investigations on the *Schismatoglottis nervosa* complex (Araceae: Schismatoglottideae) in Borneo

April Ting Pei Jen

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

ABSTRACT

This study presents biogeography hypotheses for the *Schismatoglottis nervosa* complex (Araceae: Schismatoglottideae) based on plastid (MatK) DNA sequence data. The aim is to provide insight into the floristic evolution and taxagenesis of the localized endemics in ever-wet and per-humid tropical forests. The *Schismatoglottis nervosa* complex comprises fourteen species endemic to several localities in Borneo and West Malaysia: Kuching (Bau, Matang, Padawan, and Lundu), Serian, Bintulu, Sarakei, Kapit, Miri, Tawau and Perak. Each species is endemic to specific substrate: shales, limestone, sandstone and granite. Maximum parsimony and maximum likelihood analyses were performed with PAUP* 4.0b10 using PaupUp graphical interface. Results from these analyses revealed that there are three major clades: the North East Borneo (above Lupar Line) clade, the Rejang clade, and the West Borneo clade (below Lupar Line). The results suggested that evolution was estimated to have occurred during Tertiary period. The results also indicated that the species have adapted to their own heterogeological and homogeological localities.

Key words: Biogeography, endemic, MatK data, Araceae, *Schismatoglottis nervosa* complex.

ABSTRAK


Kata kunci: Biogeografi, endemik, data MatK, Araceae, kompleks *Schismatoglottis nervosa*
CHAPTER 1

INTRODUCTION

1.1 General

1.1.1 Araceae – an introduction

Araceae is the third largest monocot family after orchids and grasses, and the seventh largest of all flowering plants after Asteraceae, Fabaceae, Rubiaceae, and Lamiaceae. The Araceae, comprising ca. 121 genera and maybe ca. 6000 species are subcosmopolitan in distribution with an overwhelming percentage of species restricted to the everwet or perhumid tropics. The major tropical centres of species’ diversity are southern Central America, the Andes (notably Colombia and Ecuador), North East South America, everwet West Africa, and Sunda (notably the island of Borneo).

Araceae are all perennial plants; most are evergreen while others are seasonally dormant. Over half of all species are monoecious plants, and almost half bisexual. A small percentage is dioecious (most Arisaema and the monotypic Pothoidium). The family is characterized macro-morphologically by a spadix, usually erect but exceedingly variable, and highly variable flower lacking floral bracteoles. Bisexual flowers may be naked, but with a perigone (perianth); unisexual flowers are usually without a perigone, but exceptions occur in Zamioculcadoideae. Floral diversity in the family is extraordinary, with almost every conceivable morphological modification occurring.
1.1.2 Schismatoglottis

Schismatoglottis is characterized by constricted spathe, loosening and tightening during female and male anthesis, and serving to control the movement of pollinators. This feature is lacking in Piptospatha, Aridarum and Bucephalandra. Schismatoglottis have infructescences with enclosure of an urceolate spathe base; however, Piptospatha, Aridarum and Bucephalandra with exposed infructescences (Bogner & Hay, 2000). Most Schismatoglottis are mesophytic chamerophytes; however, most Apoballis are nanophanerophytes or climbing and herbaceous usually mesophytic phanerophytes (P. C. Boyce, personal communication, April 23, 2010).

The Schismatoglottis nervosa complex is defined by the aromatic vegetative tissues (terpenoids) when crushed, longitudinally ribbed petioles, and leaf laminae with tessellate tertiary venation (Boyce, 2007). Schismatoglottis nervosa is characterized by longitudinally ribbed petiole, minute and punctate stigma, lacking sterile interstice. It is endemic to the South West of Sarawak, growing in association with limestone formations in the Bau area (Hay & Yuzammi, 2000).

Currently, there are two further species that belong in this complex: Schismatoglottis brevicuspis Hook. f. and Schismatoglottis elegans A. Hay. Schismatoglottis brevicuspis Hook. f. is distinguished by bright green upper part spathe, hardly opening, semi.persistent, and subcylindric spadix with contiguous fertile zone. The species is found in West Malaysia, where it is restricted to granite along rivers in lowland rain forest. Schismatoglottis elegans is
endemic to the Niah Limestones in North West of Sarawak and is defined by almost elliptic leaf blade, noticeable with petioles without minute hairs, coriaceous acuminate spathe limb, well defined sterile interstice covered with neuter structures, and with smaller appendical staminodes (Hay & Yuzammi, 2000).

1.2 Problem statement

The Schismatoglottis nervosa complex comprises 14 species, of which 11 are novel. Morphological definitions of the taxa as outlined above overlie a complex series on local endemisms, seemingly linked to one or more speciation radiations and numerous vicariance events. By understanding these radiation events, and investigating correlations between radiation events, current phytogeology, and the temporal and spatial nature of those expressed phytogeographical signatures in the context of an established phylogeny will provide insight into taxagensis in everwet and perhumid megathermal forests.

1.3 Objectives

The objective of the study is to investigate the relatedness of taxa within the Schismatoglottis nervosa complex, to correlate phytogeographical taxon patterns with the phylogeny of the complex and to present a hypothesis of taxagensis of localized endemics in everwet and perhumid megathermal forests.
CHAPTER 2
LITERATURE REVIEW

2.1 Taxonomy of the *Schismatoglottis nervosa* complex

*Schismatoglottis nervosa* complex belongs to the *Schismatoglottis asperata* group in Schismatoglottideae tribe, Aroideae subfamily of Araceae Family. Currently, the Araceae family has seven subfamilies with two in major group Proto-Araceae (Subfamily Gymnostachydoidea Bogner & Nicolson and Subfamily Orontioideae Mayo, Bogner & P.C. Boyce) and five in major group True Araceae (Subfamily Pothoideae Engler, Subfamily Monsteroideae Engler, Subfamily Aroideae, Subfamily Lasioideae Engler and Subfamily Calloideae Endlicher) (Mayo, Bogner & Boyce, 1997).

Schismatoglottis is divided into six informal groups: Corneri group, Calyptrata group, Asperata group, Multiflora group, and Tecturata group, of which four can be found in Sarawak. The Asperata Group is by far the most heterogeneous of the informal groups proposed by Hay & Yuzammi (2000) and comprises a number of distinct morphotaxa, termed complexes, including the asperata complex, barbata complex, crypta complex, hottae complex, nervosa complex, patentinervia complex, and multinervia complex, among others (P. C. Boyce, personal communication, June 7, 2009).

2.2 Geological Terrain of Sarawak

Figure 1 General locations of the complex. Retrieved 2010, April 13 from http://www.expatkl.com/gmap_mal4.html
There are two subdivisions to categorize the geological terrain of Sarawak: West Borneo Basement Block (Kuching Zone and Sibu Zone) and Eastern Borneo (Miri Zone) (Haile, 1974, as cited in Hutchison, 1989). The three zones are divided by Lupar Line and Bukit Mersing Line. The oldest rock in the West Borneo Basement Block is the pre-Moscovian known as Crystalline Schists, composed of the mica schist (van Bemmelen, 1970, as cited in Hutchison, 1989).

2.2.1 Kuching Zone

The oldest formation in Kuching Zone is the Terbat Formation, composed of limestone, chert and shale. Late Carboniferous to Early Permian fusuline fauna is found in the strata of the formation (Cummings, 1962). The Sadong Formation which lays above the Terbat Formation is composed of dark shale, conglomerate, mudstone, arkosic sandstone and tuff. The conglomerate is mostly of chert, metamorphic quartz, vein quartz and granite. The Sadong Formation is of Late Triassic era (Pimm, 1965).

2.2.2 Serian

The Serian Volcanic Formation from Kuching towards Serian was formed in the Late Triassic period. Lava near Serian contains calc-alkaline to high-K calc-alkaline basaltic andesite and andesite (Kirk, 1968). The Kedadom Formation (Wilford & Kho, 1965) which
lays over the Serian Volcanic Formation is of Upper Jurassic age and has massive sandstones, conglomerate, thin shales and limestone beds with some tuff. The conglomerate under the Kedadom Formation composed of the debris from the Serian Volcanic Formation (Hutchison, 1989).

2.2.3 Bau

The Bau limestone formation is a continuation of the Kedadom formation which overlies the Serian Volcanic Formation (Hutchison, 1989). The Bau Limestone Formation contains fossiliferous limestone, argillaceous limestone, thin layers of calcareous sandstone, and conglomerate (Wilford & Kho, 1965). Bau limestone formation contains Upper Jurassic fossils made up of micrite formed in shallow warm seas. The lower part of the limestone also contains Upper Jurassic aged fossils and consists of the Krian Member (sandstone with shale and argillaceous limestone) (Hutchison, 1989).

2.2.4 Padawan

The Padawan Formation has marine shale with subordinate sandstone and tuff, argillaceous limestone and radiolarite, indicating they are of the Lower Cretaceous age; the upper horizon of the formation contains pelagic foraminifera (Hutchison, 1989).
2.2.5 Lundu

In Lundu, the Plateau Sandstone Group (Silantek Formation, Kayan Sandstone and Plateau Sandstone) is predominantly Eocene to Oligocene and contains argillaceous member comprising thin siltstones with fine sandstones and rare conglomerates (clasts of chert and granitoids) while the upper part of the conglomerates contains current bedded sandstones with thin interbedded mudstones (Hutchison, 1989). Upper Cretaceous aged pollen flora and Upper Eocene aged radiolarian and marine mollusca are commonly found in the Plateau Sandstone Group (Wolfenden & Haile, 1963).

2.2.5.1 Gunung Gading

Gunung Gading is made up of granitic rocks with some folded sedimentary rocks from the Serabang Formation, which are the oldest rocks. The Serabang Formation also contains shales and slates, sandstones and conglomerate. There are cherts under the slate and radiolaria of Jurassic to Cretaceous age have been discovered. The granitic rocks of Gunung Gading also contain gabbro (Hazebroek & Abang Kashim, 2000). Kirk (1968) suggested that the gabbro of Early Cretaceous age. There is also Upper Cretaceous to Lower Eocene aged sandstone, conglomerate and some interbedded shale lying on the upper part of the Serabang Formation (Hazebroek & Abang Kashim, 2000).
2.2.5.2 Kubah National Park

Gunung Serapi contains sandstone conglomerate and interbedded shale from the Plateau Sandstone Formation (Hazebroek & Abang Kashim, 2000). The fossils pollen in the north and east of Gunung Serapi are of Cretaceous age (Wolfenden & Haile, 1963). There are also hard dark grey or blue shales, siltstones and some limestone from the Bau Formation; the rocks are of Cretaceous age. Conglomerates are composed of sandy rounded pebbles. Igneous rocks and metamorphic rocks are also found intruding the Gunung Serapi's sandstones, notably at the main waterfall in Kubah N.P., which consists of a substantial diorite intrusion (Hazebroek & Abang Kashim, 2000).

2.2.6 Miri Zone

The zone contains shallow marine formations where the outcropping basement is formed of the Long Bawan and Kelalan formations. Long Bawan Formation contains Late Cretaceous to Eocene aged coarse-grained lenticular sandstone and coal beds. The Late Cretaceous to Eocene-Kelalan Formation contains sandstone with hard grey shale and limestone. The Mulu Formation contains Palaeocene to Eocene miogeoclinal (non volcanic) formation of sandstone, shale and slate (Hutchison, 1989). North of Mulu Formation, the Melinau Limestone contains pure limestone from Upper Eocene to Lower Eocene (Adams, 1965, as cited in Hutchison, 1989). The Kelabit Formation contains mudstone, sandstone, with some impure limestone and
lignite. Fossils aged from Lower Oligocene to Lower Miocene were also discovered (Hutchison, 1989).

2.2.6.1 Niah National Park

Gunung Subis is composed of limestone from the Tangap Formation. The southern part consists of sandstone from the Nyalau Formation. The Subis Limestone was formed by reefs, coralline algae and tiny shellfish about 23 million years ago. The reefs were then covered by the shales and marls of the Setap Formation (Hazebroek & Abang Kashim, 2000).

2.3 Geological Terrain of Sabah

In South Western Sabah, the Upper Cretaceous to Upper Eocene Sarawak Mentarang formation continues into Sabah and is known as the Sapulut Formation. It is made up of argillaceous and thin siltstone beds of typical distal turbidite aspect. North of Sapulut Formation, the Eocene Trusmadi Formation contains argillite and, siltstone layers of distal turbidite aspect. Besides, there are also metamorphised phyllite, quartz and limestone breccias (Colenette, 1965). In North West Sabah, the West Crocker Formation also contains argillaceous with thin siltstones. This formation continues into Beaufort and the Klias Peninsula to become the Oligocene to Lower Miocene Temburong Formation also with argillaceous with thin siltstone layers (Wilson, 1964). Tawau contains rhyolite, acidic and
basalt volcanic rocks of Miocene. Intrusion of basic to intermediate dolerite of early Pliocene age is also found in the area (Fox, 2005).

2.4 Geological Terrain of Peninsular Malaysia

There are two geological terrains in Peninsular Malaysia: the Eastmal Block (Central and Eastern Belt of Peninsular Malaysia) (Hutchison, 1989) and Western Belt of Peninsular Malaysia (Azman, 2000). Peninsular Malaysia is divided by Raub-Bentong suture into the Sibumasu block (Western Belt) and Eastern part of Peninsular Malaysia (Central and Eastern Belt). Hutchison (1989) stated that Eastern Belt of Peninsular Malaysia was once attached to Indochina before it was displaced during Late Mesozoic.

Sibumasu (part of Peninsular Malaysia, Thailand and Sumatera) originated in Australia. The block is believed to have drifted from Australia before colliding with Indochina and Eastern part of West Malaysia during the late Permian (Melcalfe, 1990, as cited in Harbury, Jones, Audley-Charles & Mohamed, 1991). Hutchison (1989) stated that Eastmal contains Cretaceous granites and syenites. The sediments also contain rhyoactic and andesites from Carboniferous to Cretaceous volcanism. The Western Belt of Peninsular Malaysia consists of granites with an age range from 200 to 230 Ma while the Eastern and Central Belt consists of granite with an age range from 200 to 294 Ma (Cobbing et al., 1992, as cited in Azman, 2000)
2.5 Cladistics

Cladistics is a method that systemizes taxa according to their phylogenetic relationships. Cladistics can be used to assume biogeography and studies of co-evolution. The phylogenetic relationship corresponds to the common ancestry of the taxa and only exists between distinct species or supraspecific taxa (Kavanaugh, 1978). Molecular phylogeny reconstruction is a cladistic method used to assume the phylogenetic relationships between taxa by using molecular data. The amount of molecular data is large and is simple to obtain the data in the laboratory. These molecular data are used to identify the nucleotide base at an informative site, thus can be used to compare taxa with the most diverse characteristics (Kumar & Filipski, 2008). Morphological data is also used in phylogeny reconstruction to resolve the relationships between fossil taxa and living taxa (Wiens, 2004).

2.5.1 Maximum Parsimony phylogeny generation

Maximum parsimony (MP) has been the most popular method used to reconstruct phylogenetic trees. Maximum parsimony is based on the highly contentious assumption that "evolution is parsimonious" (Jin, Nakhleh, Snir & Tuller, 2007). MP is a non parametric method which searches for the minimum number of character state change without considering the parameters for each tree topology (Kolaczkowski & Thornton, 2004). The tree is reconstructed by minimizing the total number of mutation (substitution) events along the
tree branches (Jin, Nakhleh, Snir & Tuller, 2007). An overwhelming advantage of maximum parsimony is that it reduces computational time and, at least in theory, limits the number of final trees.

Maximum parsimony performs well under a wide range of conditions including moderate heterogeneity (Kolaczkowski & Thornton, 2004). With the summation of the numbers of character state changes, the topology with the smallest total is taken into account to reconstruct the tree (Yang, 1996). Jin, Nakhleh, Snir & Tuller (2007) stated that maximum parsimony can be used to detect horizontal gene transfer (HGT). HGT events can be detected by using the maximum parsimony criterion and is required to map the evolutionary history of a data set onto the species phylogeny. However, maximum parsimony will produce misleading topologies if the total of character state changes is large (Strimmer & Haeseler, 1996). If non-parsimonius evolution (e.g., leap-frog speciation; nothospecies events, non steady-state evolution, etc.) are suspected other tree-generating algorithms should be considered parallel to MP.

2.5.2 Maximum Likelihood phylogeny generation

Maximum Likelihood (ML) is also one of the most popular methods for reconstructing phylogenetic trees. It is a parametric method which determines a tree topology and branch length for the tree topology. ML uses heuristic searches to hunt for the best tree topology. The
number of tree topologies will increase exponentially with the number of sequences. Because of the process of the optimization of branch length, maximum likelihood analysis usually takes more time compared to the MP analysis (Strimmer & Haeseler, 1996). Through ML, branch length is adjusted until the probability of the data set is the highest (Lewis, 1998). ML also allows estimation of the evolutionary rates by likelihood ratio tests and provides a rough indication of the error of the estimate of the tree (Felsenstein, 1981).

2.5.3 Gene region: MatK

The matK gene is a protein coding region located in the trnK intron; it is one of the most rapidly evolving genome regions. It is approximately 1550 bp and may be involved in the splicing of the type II introns for RNA transcripts (Neuhaus & Ling, 1987; Ems et al., 1995, as cited in Soltis & Soltis, 1998). The matK gene is used to investigate the intergeneric and interspecific relationships in seed plants (Soltis & Soltis, 1998). The gene is easily amplified using universal primers because the trnK intron is near to psbA in terms of location (Hilu et al., 2003). There are sequencing primers developed for the gene such as 1412F, 1470R, and 1235R, but the design of additional sequencing primers is still required for different kinds of study interest (Lewis, 1998).