SYSTEMATIC STUDIES OF GIAMENSIS AND HANNEAE COMPLEXES (*HOMALOMENA*: HOMALOMENEAE: ARACEAE)

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SYSTEMATIC STUDIES OF GIAMENSIS AND HANNEAE COMPLEXES

(HOMALOMENA: HOMALOMENEAE: ARACEAE)

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2012
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.

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(Homalomena Schott is one of the most abundant, speciose, least understood aroid genera in Tropical Southeast Asian. Taxonomy, pollination investigations, floral odour analyses and phylogeny of six Homalomena species in Hanneae and Giamensis Complexes were investigated in this study. Three new species- Homalomena gastrofructa sp. nov. ined. Y.C.Hoe, S.Y.Wong & P.C.Boyce, H. velutipedunculata sp. nov. ined. Y.C.Hoe, S.Y.Wong & P.C.Boyce and H. baangongensis sp. nov. ined. L.S.Tung & Y.C.Hoe were discovered. The main pollinator for all the seven Homalomena species is Parastasia spp. (Scarabaeidae: Rutelinae). Chrysomelids (Chaleonus schawalleri, Chaleonus spp. & Deceptina sp. (Coleoptera: Chrysomelidae) are the opportunist pollinators. Colocasiomyia (Diptera: Drosophilidae), Trigona sp. (Apinae: Meliponini), unidentified earwigs (Dermaptera), unidentified pale yellow and orange chrysomelids (Coleoptera: Chrysomelidae), unidentified Nitidulidae and Staphylinidae are merely visitors. The food reward interpistillar staminode is the main pollinator attraction. Pollinator Parastasia spp. that consumed the interpistillar staminode is regarded as highly mutualistic between Parastasia-Homalomena. During pistillate anthesis (Hanneae Complex) floral odour serves to attract and retain insect pollinator until the end of the anthesis by remitting second less intensified odour during staminate anthesis. Floral odour serves to attract insect pollinator only in pistillate anthesis (Giamensis Complex) but did not or weakly remitted flora odour during staminate anthesis. Gas chromatography-mass spectrometry (GC-MS) analyses reveal three compounds: Cyclohexane,2-ethenyl-1,1-dimethyl-3-methylene-, Cyclohexane,4-methyl-2-methylene-1-(1-methylethylidene) and Cyclohexene, 4-methyl-3-(1-methylethylidene)- and these have never been reported in Araceae. Hanneae Complex species can
be represented by 1) majorly produce Terpenoid compounds especially alpha-pinene). Whereelse Giamensis Complex species can be differentiated by 1) emitting majority compound of Acetic Acid, butyl ester and Furan, 3-(4-methyl-3-pentenyl)- but absent during staminate anthesis. Phylogeny was constructed for 17 taxa of all the complexes in Cyrtocladon, Homalomena, Punctulata and Chamaecladon Supergroups based on the nuclear internal transcribed spacer. The aligned sequences were analysed in PAUP* 4.0b10 for maximum parsimony and 'Advanced Mode' analyses for maximum likelihood. The tree supported delimitation of Hanneae and Giamensis Complexes from taxonomy, pollination, floral odour were mapped onto the phylogenetic tree to provide further evidences on the separation of both Complexes. This study provided an indepth investigation to explain the high level of biodiversity in the genus *Homalomena* in Borneo.

Key words:

Floral odour, Giamensis Complex, *Homalomena*, Hanneae Complex, internal transcribed spacer, pollination, molecular phylogeny, taxonomy.
Kajian Sistematik Homalomena Giamensis Kompleks dan Homalomena Hanneae Kompleks

(Homalomena: Homalomeneae: Araceae)

ABSTRAK

pistillate antesis (Giamensis Kompleks) dengan tidak atau mengeluarkan bau bunga yang nipis semasa staminate antesis. Analisis gas chromatography-mass spectrometry (GC-MS) mendedahkan tiga kompound: Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-, Cyclohexane, 4-methyl-2-methylene-1-(1-methylethylidene)- dan Cyclohexene, 4-methyl-3-(1-methylethylidene)- dan kompaun tersebut belum pernah dilapor di Araceae. Hanneae Kompleks spesies boleh diwakili dengan 1) menghasilkan Terpenoid kompound yang banyak terutamanya Alpha-Pinene 2). Manakala Giamensis Kompleks spesies boleh dibezakan dengan 1) mengeluarkan kompound utama Acetic Acetic acid, butyl ester and Furan, 3-(4-methyl-3-pentenyl)- tetapi kompound tersebut tiada di staminate antesis. Filogeni bagi 17 taksa daripada semua Kompleks di Cyrtocladon, Homalomena, Punctulata dan Chamaecladon Supergroups dibina berdasarkan nuklear internal transcribed spacer. Susunan rantaian-rantaiandianalisis dengan menggunakan PAUP* 4.0b10 bagi maksimum parsimony dan ‘Advanced Mode’ analisis bagi maksimum likelihood. Pokok menyokong delimitation Hanneae dan Giamensis Kompleks dari segi taksonomi, pendebungaan, bau bunga yang digabung ke pokok filogenetik untuk membekalkan bukti bagi pengasingan kedua-dua Kompleks terbabit. Kajian ini membekalkan satu kajian dalaman bagi menerangkan biodiversity yang tinggi bagi genus Homalomena di Borneo.

Kata kunci:
Bau bunga, Giamensis Kompleks, Homalomena, Hanneae Kompleks, internal transcribed spacer, pendebungaan, molekul filogeni, taksonomi.
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<tr>
<td>PAUP</td>
<td>Phylogenetic Analysis using Parsimony</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>MC</td>
<td>Mass spectrum</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography-mass spectrometry</td>
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<tr>
<td>ITS</td>
<td>Internal Transcribed Spacer Region</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reactions</td>
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<tr>
<td>°C</td>
<td>Degree Celsius</td>
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<td>M</td>
<td>Meter</td>
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<td>M</td>
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<td>mm</td>
<td>Milimeter</td>
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<td>Um</td>
<td>Micrometer</td>
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<td>mM</td>
<td>Millimolar</td>
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<td>uL</td>
<td>Microliter</td>
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<td>%</td>
<td>Percent</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyl Trimethyl Ammonium</td>
</tr>
<tr>
<td>dNTPs</td>
<td>Deoxynucleotide triphosphates</td>
</tr>
<tr>
<td>Taq DNA polymerase</td>
<td><em>Thermus aquaticus</em> DNA polymerase</td>
</tr>
<tr>
<td>MgCl2</td>
<td>Magnesium chloride</td>
</tr>
<tr>
<td>PVP</td>
<td>Poly Vinyl Pyrrolidone</td>
</tr>
<tr>
<td>TBR</td>
<td>Tree-bisection-reconnection</td>
</tr>
<tr>
<td>aLRT</td>
<td>Approximate Likelihood-Ratio Test</td>
</tr>
<tr>
<td>Bp</td>
<td>Base pair</td>
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<tr>
<td>MP</td>
<td>Maximum Parsimony</td>
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<tr>
<td>ML</td>
<td>Maximum likelihood</td>
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<tr>
<td>CI</td>
<td>Consistency index</td>
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<td>Homoplasy index</td>
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<tr>
<td>RI</td>
<td>Retention index</td>
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<tr>
<td>RCI</td>
<td>Rescaled consistency index</td>
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1.1 Araceae-General

The Araceae (colloquially aroids), is a highly diverse family of monocotyledons comprising 119 genera and estimated 5500 species (Boyce & Croat, 2011). They are diverse in hemiepiphytes, epiphytes, litophytes, geophytes, rheophytes, helophytes, submerged or free-floating aquatics; mostly concentrated in tropic Southeast Asia, Malay Archipelago (notably Borneo), tropic southern Central America and West Africa. Most Araceae are evergreen, others are seasonally dormant and all are perennial. More than half of the genera have unisexual flower (Aroideae), others bisexual ones (Gymnostachydoideae, Orontioideae, Pothoideae, Monsteroideae, Lasioideae, Calloideae). Unisexual inflorescence has spathe differentiation into spathe limb and lower spathe zone; spadix differentiation into pistillate and staminate zone. Bisexual inflorescence is simple, naked, with spreading spathe and lacking zone differentiation (spathe and spadix) (Mayo et al., 1997).

1.2 Homalomena Schott

The tribe Homalomeneae comprises two accepted genera: Homalomena Schott and Furtadoa M. Hotta. Furtadoa has only two described species [Furtadoa sumatrensis M. Hotta and Furtadoa mixta (Ridl.) M. Hotta] and differs from Homalomena by having each staminate flower associated with a pistillode. Furtadoa is only found on Sumatera (Indonesia) and in Peninsular
Homalomena is estimated to have more than 500 species (Boyce & Croat, 2011; Ng et al., 2011; Wong & Boyce, 2011), the majority undescribed (Boyce & Wong, 2008; Boyce et al., 2010; Ng et al., 2011, Wong & Boyce, 2011) distributed in tropical and sub-tropical Asia, with a few species in the Neotropics (Mayo et al., 1997), with the three main centres of diversity: Sumatera, Borneo, and New Guinea (Boyce et al., 2008). To date, only 30 accepted published names are available for Bornean Homalomena, of which 16 have been recently described (Boyce & Wong, 2008; Baharuddin & Boyce, 2010; Boyce et al., 2010; Tung et al., 2010; Hoe et al., 2011b; Kurniawan et al., 2011; Wong & Boyce, 2011). Pending phylogenetic analyses, Asian Homalomena is divided into four informal morphotaxa Supergroups (Cyrtocladon, Chamaecladon, Homalomena and Punctulata [Hotta’s Geniculatae- Ng et al., 2011]) based on the morphological evidence of vegetative and reproductive structures (Boyce et al., 2008; Ng et al., 2011). Ng et al. (2011) and Wong & Boyce (2011) further divided the Cyrtocladon Supergroup into seven complexes: Borneensis, Giamensis, Hanneae, Havilandii, Insignis, Rostrata, and Wongii Complex.

Taxonomical work on Homalomena is difficult as many historical type specimens are poorly preserved. Despite the abundance of Homalomena specimens in herbaria, the majority of specimens are either undetermined, or carry incorrect determinations. Indeed, much of the material is effectively undeterminable owing to: (1) post-preservation depredations by beetles,
and (2) collected post-anthesis by which time critical floral morphologies, notably interpistillar staminodes, have been irreparably damaged during pollination. However, provided concise locality data are available, it is easy to re-visit key localities, and prepare adequate samples (images, inflorescences in alcohol) for any suspected novelties (Boyce & Wong, 2008).

1.3 Pollination

Pollination in plant-pollinator interaction has likely played a role in increasing the diversity of the angiosperm and pollinator (Campbell & Reece, 2005). In contrast to the high diversity of Araceae, only over half the accepted genera and about 4% of species have been studied (Gibernau, 2011). Previous pollination studies of Homalomena suggested Drosophilidae (flies) as the pollinator (Okada 1986; Yafuso & Okada, 1990; Sultana et al., 2006). However, several recent studies have suggested beetles (Scarabaeidae and possibly Chrysomelidae) to be a more reliable pollinator than Drosophilidae (Kumano-Nomura & Yamaoka, 2009; Tung et al., 2010; Hoe et al., 2011a). Phylogeny studies based on pollination and molecular evidence in aroid are rare except the study by Takano et al. (2011) that investigated mitochondrial NDS sequences and cohabitation breeding habit (mostly in host inflorescence of Alocasia (Schott) G. Don and Colocasia Schott) of Colocasiomyia de Meijere flies.

1.4 Floral Odour Analyses

Chemical compounds in floral odours may be analyzed through series of procedures for trapping, separating (Gas Chromatography (GC), identifying, and analyzing (Mass Spectrum (MS) (Tholl & Röse, U.S.R., 2006). Floral odour analyses have been well investigated in genus Arum L.
Quilichini et al., 2010; Urru et al., 2010; Chartier et al., 2011), Anthurium Schott (Kuanprasert et al., 1998; Schwerdtfeger et al., 2002) and Amorphophallus Blume ex Decaisne (Kite, 1995; Kite & Hetterscheid, 1997; Kite et al., 1998). These analyses provide significant data in species boundary delimitation (Dodson et al., 1969; William & Whitten, 1999) especially angiosperms with highly similar morphology (Knudsen et al., 1996), growing sympatrically, synchronous flowering and having similar pollination mechanism (Knudsen et al., 1996). Studies have verified the role of a specific or blend of chemical compounds in attracting the specific pollinators such as Psychoda (Diptera, Psychodidae) was attracted to three major compounds (p-cresol, indole and 2-Heptanone) emitted by Arum maculatum L. (Kite et al., 1998) but the mixture of these three compounds was the most potent. Primary pollinator (Parastasia bimaculata- Scarabaeidae: Rutelinae) and a secondary pollinator (Chaleonus schawalleri- Chrysomelidae) were attracted to compound blends of 2-butanol and veratrole of an undescribed Homalomena (erroneously identified as Homalomena propinqua Schott (Boyce, pers. comm.) (Kumano-Nomura & Yamaoka, 2009).

1.5 Problem Statements

Homalomena is by far the most speciose aroid genus in Indomalaya, with a very significant proportion of the species yet to be formally described (Boyce & Croat, 2011). Frequently, several species from either supergroups or complexes occur sympatrically, often flowering synchronously and this warrant further investigations in plant-pollinator interaction to provide evidence(s) for species delimitation. Previous works on pollination observations in Homalomena reveal Drosohophilidae (Okada 1986; Yafuso & Okada, 1990; Sultana et al., 2006), Scarabaeidae
(Kumano & Yamaoka, 2006; Kumano-Nomura & Yamaoka, 2009; Tung et al., 2010; Hoe et al., 2011a) or Chrysomelidae (Kumano & Yamaoka, 2006; Hoe et al., 2011a) as pollinators. Floral odour of *Homalomena* was only investigated on a single species by Kumano-Nomura & Yamaoka (2009). A phylogenetic framework is crucially needed to determine the phylogenetic relationships for the studied taxa and validate the species (Complexes) delimitations. Secondly to be able to interpret within an evolutionary context the changes in morphology, pollination and floral odours among the studied species. Lastly to provide a platform to tie in data on taxonomy, pollination and floral odour and these provide a true relationship to justify species delimitation among the investigated taxa.

1.6 Objectives

Seven species from the Hanneae complex (*Homalomena debilicrista* Y.C. Hoe, S.W. Wong & P.C. Boyce, *Homalomena gastrofructa* sp. nov. ined. Y.C. Hoe, S.Y. Wong & P.C. Boyce and *Homalomena velutipedunculata* sp. nov. ined. Y.C. Hoe, S.Y. Wong & P.C. Boyce), Giamensis complex (*Homalomena matangae* Y.C. Hoe, S.Y. Wong & P.C. Boyce, *Homalomena baangongensis* sp. nov. ined. L.S. Tung & Y.C. Hoe and *Homalomena giamensis* L.S. Tung, S.Y. Wong & P.C. Boyce) and the Borneensis complex (*Homalomena borneensis* Ridl. c.f.) as the outgroup species were investigated for this study. The objectives were:

1. To delimitate species boundaries using comparative macromorphology and molecular techniques.
2. To field-observe pollination strategies and identify the pollinators.
3. To analyze floral odour compositions using gas chromatography-mass spectrometry (GC-MS).

4. To correlate pollinator activities with floral odour composition at pistillate and staminate anthesis.

5. To determine the phylogenetic relationships for the taxa investigated using nuclear full ITS region.

6. To map onto the resultant phylogenetic tree data from taxonomy, pollination and floral odour analyses.

7. To provide suggestions (s) for the rich level of diversity in *Homalomena* on Borneo based on the co-evolutionary evidence of pollinators and plant species.
2.1 Molecular Analysis using Internal Transcribed Spacer (ITS) Region in Homalomena

The nuclear ribosomal internal transcribed spacer (ITS) region is one of the most useful types of nuclear DNA sequences in plant systematics. The ITS region occurs between the 18S and 26S nuclear ribosomal DNA. Recently ITS has been increasingly utilized for phylogenetic study of the Araceae (Gauthier et al., 2008; Low et al., 2011; Wong et al., in review). Low et al., (2011) showed that the ITS is useful at low taxonomic level in the Hottarum group (Araceae: Schismatoglottideae). Wong et al., (in review) also used ITS to differentiate out clades of Neotropic Homalomena and Tropic Homalomena respectively in the Supergroups and Complexes level. Besides Araceae, the ITS also has been frequently used to determine the phylogenetic relationship among putatively closely-related species (e.g., Simpson, 2006) such as the orchid genus Stanhopea (Williams & Whitten, 1999; Whitten, et al., 2000) and has been successful to resolve interspecific relationships within Asteraceae (Mitsui et al., 2007) and Resedaceae (Martín-Bravo et al., 2007). Including of ITS with floral odour data also showed some correlated result in the phylogenetic demilitation tree of Stanhopea (Orchidaceae) (Williams & Whitten, 1999).
2.2 The History of Pollination Investigations

Dr Joseph Gottlieb Kölreuter (1733-1806) was the first to observe the flower pollination. In his *Vorläufige Nachricht von einigen das Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen* (Leipzig, 1761, 1763, 1764 & 1766) presented by Knuth (1906), Kölreuter carried out numerous hybridization experiments and concluded that flowers are necessary to be cross-pollinated by the help of insects. He discovered the stigma is only effected by the insects in all cucumbers, iris plants, and many Malvaceae. Kölreuter noted the nectar is required by the insects, and during the nectar collection, insects gathered great quantity of pollen among the hairs of their bodies, then cross-pollinated by rubbing the pollen off again on the stigma (Knuth, 1906).

Christian Konrad Sprengel (1750-1816) studied the pollination systematically (Proctor *et al.*, 1996). He lifted this branch of botany to a very high level, made clear essential ideas of the theory of flowers, highlighting structural adaptation of insect pollination of c. 500 angiosperms in his *Das entdeckte Geheimniss der Natur im Bau und in der Befruchtung der Blumen* (1793) book and many were described in great details (Knuth, 1906). For examples, Sprengel discovered the soft hairs on bases of the *Geranium sylvaticum* L. petals are chiefly to prevent the nectar being spoilt by the rain. Yellow ring mark that surrounds the opening of corolla tube (*Myosotis palustris* L.) was the honey guide mark. He also revealed deception behaviour in nectarless *Orchis latifolia* L. and *O. morio* L.. However, his works received little attention and went forgotten due to the influence of Linnaeus (1707-1778) and Linnaeus's successor that assume the real end of botany is building up the systematic botany at that time (Knuth, 1906).
By the time where Charles Darwin (1809-1882) published the Origin of Species (1859), Sprengel’s work received the recognition. Darwin mentioned Sprengel’s works and rescued Sprengel’s work from oblivion. Sprengel’s observation came very near to cross-pollination (but not expressed), later proved by the work of Darwin. Darwin prohibited insect visitation by covering some Papilionaceae with a net and it resulted in the seeds formation being not vigorous. He further showed that the most abundant formation of seedstakess place when there is pollination of the stigmas with pollen in legitimate fertilization. Darwin’s book ‘On the various contrivances by which British and foreign orchids are fertilised by insects, and on the good effects of intercrossing’ presented his work on Orchids, in which he described the adaptations for fertilization of British and foreign orchids but it is the same information that provided by Sprengel seventy years ago.

In the same century, Asa Gray (1810-1888) investigated mostly orchids in North America, subsequently cleistogamy, self-fertility and humming bird pollinated-flowers (Knuth, 1906). In South America, Fritz Müller (1821-1897) worked on adaptation pollination in Posoqueria, Heeria, humming-bird pollinated-flowers, poison-like action in self-pollination and di- and trimorphous plants in Brazil. Friedrich Hildebrand (1867) gave the first classification of floral arrangements in ‘Die Geschlechtverteilung bei den Pflanzen’ (see Knuth, 1906). Federico Delpino described more floral arrangement and adaptation into oecological groups for the whole plant kingdom. He distinguished 47 groups into 13 classes (see Knuth, 1906). Hermann Müller (1829-1883) criticised Delpino’s works to some extent quite arbitrary. For example, Delpino mentioned the 6th group (hydrangea) is supposed to adapt for Cetonia and lamellicorns but yet
flies, bees and butterflies also visited hydrangea. However, Loew (1895) mentioned Delpino
works were regarded as one of the most suggestive and brilliant attempt towards the solution of a
problem to nature according to the standpoints of science at that time.

Hermann Müller’s research stimulated other botanists to follow his work even greater than other
specialists mentioned above. His ecological context foreshadowed the rich development of
pollination ecology that will occur a century later. He firstly began floral statistics to investigate
the connection between pollination mechanism and insects’ visitors and published his three
important works between 1873-1881 (Knuth, 1906 and references therein; Proctor et al., 1996
and reference therein).

In recent decades, new interests of pollination ecology such as studies of floral traits, the
dynamics of pollen transfer, competition in pollinator service, specialization and generalization
relationships have emerged from pollination investigation (Mitchell et al., 2009). Advance
technical equipments such as visible-light and ultraviolet photography, cinematography, scanning
electron microscopy (SEM), gas chromatography-mass spectrometry (GC-MS) also have
contributed to pollination biology (Proctor et al., 1996). Evolutionary discipline through
molecular analyses was combining with ecology data (breeding habit and geographical area,
(Takano et al., 2011) to investigate the phylogenetic relationship between plant-pollinator
particularly in Asian ariods.