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Phylogeny of *Amorphophallus* (Araceae) on Borneo with notes on the floral biology of three species

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ABSTRACT: This study was undertaken on *Amorphophallus* of Borneo to address two questions: (1) to determine the phylogenetic relations among taxa of Bornean *Amorphophallus* and (2) to investigate the floral biology and floral visitors of three *Amorphophallus* species. Phylogenetic analyses were carried out by using one plastid region: *matK*, and two nuclear regions: ITS and PhyC, with a total of 98 accessions representing 56 taxa of *Amorphophallus*. Floral biology of three *Amorphophallus* species (*A. hewittii*, *A. eburneus*, and *A. julaihii*) were investigated. Bornean *Amorphophallus* is separated into three groups within subgen. *Amorphophallus*: *A. angulatus* and *A. pendulus* of the Paeoniifolius-Manta clade, *A. ranchanensis* as sister taxon to clade A, clade Pusillus II, and clade B. The anthesis of *A. hewittii* and *A. eburneus* lasted for ca. 49 hours and ca. 64 hours respectively. The pistillate anthesis was much longer in *A. hewitti* (36 hours) than *A. eburneus* (24 hours) but the staminate anthesis was much shorter in *A. hewittii* (13 hours) than *A. eburneus* (40 hours). Floral visitors to *A. hewittii* are different to those visiting *A. eburneus* and *A. julaihii*; the latter two species attract less visitors and belong to clade A where hitherto no species has been investigated.

KEY WORDS: *Amorphophallus eburneus*, *Amorphophallus hewittii*, *Amorphophallus julaihii*, Sarawak.

INTRODUCTION

Amorphophallus Blume ex Decne. the sole accepted genus of tribe Thomsonieae (Araceae) has approximately 220 species (Boyce and Croat, 2011 onwards). *Amorphophallus* comprises mainly lowland plants, growing in the tropical and subtropical zones of the Paleotropics from West Africa to the Pacific Islands and Japan (Mayo *et al.*, 1997) with the centre of diversity in IndoMalaya (Boyce and Croat, 2011). Borneo has 19 indigenous species, all are endemic. Ten species occur in Sarawak: *A. angulatus* Hett. & A. Vogel, *A. brachyphyllus* Hett., *A. eburneus* Bogner, *A. hewittii* Alderw., *A. infundibuliformis* Hett., A. Dearden & A. Vogel, *A. julaihii* Ipor, Tawan & P.C. Boyce, *A. juliae* P.C. Boyce & Hett., *A. niahensis* P.C. Boyce & Hett., *A. pendulus* Bogner & Mayo, and *A. ranchanensis* Ipor, A. Simon & Meekiong and four species in Sabah: *A. lambii* Mayo & Widjaja, *A. rugosus* Hett. & A.L. Lamb, *A. tinekeae* Hett. & A. Vogel, and *A. venustus* Hett., A. Hay & J. Mood. One species, *A. hottae* Bogner & Hett. occurs in Sarawak and Sabah (Boyce *et al.*, 2010; Ipor *et al.*, 2012). *Amorphophallus borneensis* (Engl.) Engl. & Gehrm., *A. costatus* Hett., *A. linguiformis* Hett., and *A. suwidjianus* Ipor, Tawan & Meekiong are known only from Kalimantan.

Amorphophallus is supported as a monophyletic with the inclusion *Pseudodracontium* N.E. Br. (Hetterscheid and Claudel, 2012; Claudel *et al.*, 2017). The first analyses of species-level relationships in *Amorphophallus* were based on limited sampling (ca. 30% of species diversity) (Grob *et al.*, 2002, 2004; Sedayu *et al.*, 2010) and revealed a small number of well-supported clades, among which the relationships were unresolved. Claudel *et al.* (2017) expanded the taxonomic sampling to include 70% of the known species using nuclear (ITS1) and plastid (*rbcL* and *matK*) gene regions. Their analyses resolved four clades treated as subgenera: *Amorphophallus*, *Metandrium* Stapf, *Scutandrium* Hett. & Claudel, and *Afrophallus* Hett. & Claudel. Subgenus *Amorphophallus* is also termed as the South East Asia (SEA) clade and comprises taxa distributed from India eastwards via continental South East Asia and Indonesia to the Philippines and Australia (Claudel *et al.*, 2017).

The pollination biology of *Amorphophallus* is hitherto known from a few field observations (van der Pijl, 1937; Bogner, 1976; Sivadasan and Sabu, 1991; Beath, 1996; Hetterscheid and Ittenbach, 1996; Singh and Gadgil, 1996; Punekar and Kumaran, 2010; Moretto *et al.*, 2019; Tang *et al.*, 2020) and have confirmed pollinators as: beetles (Cetoniidae, Nitidulidae, Scarabaeidae, Silphidae, and Staphylinidae), dung flies (Platystomatidae), and bees (*Trigona*). Apart from these pollinators, *Amorphophallus* species are also visited by beetles (Bostrichidae, Brentidae, Histeridae, Hybosoridae, Lyctidae, Rutelinae), flies (Calliphoridae, Drosophilidae, Muscidae), ants (Formicidae, Dolichoderinae), cockroaches (Blaberidae/Panesthiinae), and spiders (Sivadasan and Sabu, 1991; Hetterscheid, 1995; Hetterscheid and Ittenbach, 1996; Giordano, 1999; Punekar and Kumaran, 2010).

There are 19 indigenous *Amorphophallus* occurring on Borneo and to date, there is yet a study involving the inclusion of these taxa into a phylogeny which will serve

as a prerequisite to allow better understanding on the evolutionary context of the Bornean *Amorphophallus*. As observations of in situ plant-pollinator interactions are very much lacking in Borneo, we provide observations on three *Amorphophallus* species. Thus, this study was undertaken to address two main questions: (1) to determine the relatedness among Bornean *Amorphophallus* taxa and (2) to investigate the floral biology and floral visitors of three Bornean *Amorphophallus* species (*A. hewittii*, *A. eburneus*, and *A. julaihii*).

MATERIALS AND METHODS

Plant material

Evolutionary relationships of *Amorphophallus* on Borneo were inferred by analysing sequence data from one plastid region, *matK* and two nuclear regions, ITS and PhyC, of 98 accessions of 56 *Amorphophallus* species. Fourteen taxa (46 accessions) of the 19 known taxa (70%) from Borneo and 42 taxa (52 accessions) from outside Borneo were included in the analyses. *Amorphophallus costatus* and *A. infundibuliformis* were not included in previous phylogenetic studies. The sampling represented all clades of subg. *Amorphophallus*: Paeoniifolius-Manta clade, Pulchellus-clade & Pusillus-clade (Claudel *et al.*, 2017). Outgroups were: *Syngonium auritum* Schott and *Chlorospatha longipoda* (K. Krause) in line with Cusimano *et al.* (2011). One hundred and twenty-three sequences representing 46 plant individuals were newly generated in this study and deposited in GenBank with the accession number from KY490411 to KY490533. One hundred and two sequences were downloaded from NCBI: 50 sequences of ITS1 and 51 sequences of *matK* from Claudel *et al.* (2017), and one sequence of PhyC from Chartier *et al.* (2014). Voucher information and GenBank accession numbers for all taxa are provided in the supplement Table S1. Data matrices were deposited to TreeBASE (study number S21456: http://purl.org/phylo/treebase/phylows/study/TB2:S21456).

DNA extraction, amplification and sequencing

Fresh leaf samples were collected during fieldwork and stored separately in silica bags. Total DNA was extracted using a modified version of the 2×CTAB protocol (Doyle and Doyle, 1987) with the addition of PVP (Polyvinylpyrrolidone; Wong *et al.*, 2010). ITS1, 5.8S subunit and ITS2 were amplified using the primer pairs 18F and 26R (Käss and Wink, 1997, modified after Beyra-Matos and Lavin, 1999). Polymerase chain reaction (PCR) amplifications for *matK* were carried out using the forward primer 19F (Gravendeel *et al.*, 2001) and reverse primer 2R (Steele and Vilgalys, 1994). Two internal primers, 390F or/and 1236R (Cuénoud *et al.*, 2002) were used for sequencing. Four primers: A20F, 430F, 748R, and AR were used for PhyC (Nauheimer *et al.*, 2012). PCR amplifications and protocols for all regions followed Low *et al.* (2011) with the cycles (denaturation, annealing, and elongation steps) reduced to 30 cycles. PCR products were run on 1.0-2.0% agarose gel premixed with 0.05% SYBR® Safe DNA gel stain (Invitrogen, Eugene, Oregon, U.S.A.). PCR products were sent for sequencing in forward and reverse directions using Sequencer ABI 3730xl at BGI Tech Solutions (Hong Kong) Co., Limited, Hong Kong.

Sequence alignment and phylogenetic analyses

Newly generated sequences for both regions were manually trimmed and assembled for each taxon. These sequences were combined with previously generated sequences for each region. The data matrices were aligned using MUSCLE (Edgar, 2004) as implemented in Geneious Pro v5.6.4 (Biomatters Ltd., Auckland, New Zealand; www.geneious.com; Drummond *et al.*, 2012) followed by minor manual adjustment following the similarity criterion (Simmons, 2004). Indels were treated as missing data. To infer phylogenetic relationships, we applied maximum likelihood (ML; Felsenstein, 1985) optimization with the software RAxML (7.3.2; Stamatakis, 2006) and RAxML- Gui (Silvestro and Michalak, 2012), as well as a Bayesian approach (Yang and Rannala, 1997) with the software MrBayes (3.2.1; Huelsenbeck and Ronquist, 2001). The ML analyses were performed using the generalized time-reversible substitution model with gamma rate heterogeneity and statistical support was accessed via 1,000 replicates (repeated 10 times). The Bayesian analyses were performed using General Time Reversible plus Invariable Sites plus Gamma (GTR+I+G, for ITS and *matK*) and Transversion Model plus Invariable Sites plus Gamma (TVM+I+G, for PhyC), as identified by the Akaike information criterion (AIC; Akaike, 1974) in FindModel (http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html). Statistical support was accessed via posterior probability (PP). Markov chain Monte Carlo (MCMC) analyses were conducted twice to check for parameter convergence. The MCMC algorithm was run for 10,000,000 generations with one cold and three heated chains, starting from random trees and sampling one out of every 100 generations. Convergence was assessed by using the standard deviation of split frequencies as convergence index with values < 0.02 interpreted as indicating good convergence. The first 10% of trees were discarded as burn-in. Remaining trees were used to construct 50% majority-rule consensus trees. Throughout this paper, PP of 0.9–0.95 support and ML bootstrap (BS) value of 70–84% are considered as moderate support, a PP of 0.95–1 and BS value of 85–100% are considered as strong support.

Floral biology

Three species were selected in this study: *A. hewittii*, *A. eburneus*, and *A. julaihii*. We chose to investigate the populations of *A. hewittii* and *A. eburneus* at Kg Danu, Padawan, Kuching Division, Sarawak, Malaysian Borneo (01°17'07.2"N, 110°15'08.8"E, 40 m elevation). *Amorphophallus hewittii* occurred next to *A. eburneus* on the limestone. The population of ca. 25 plant individuals of *A. hewittii* grew in deep leaf litter over limestone-derived loams on ridges and moderate slopes beneath lowland perhumid evergreen broadleaf forest adjacent to a karst limestone. The population of *A. eburneus* was much larger, ca. 300 plant individuals, occurring on the cliffs and boulders of the limestone. The investigation on *A. julaihii* was carried out at the karst limestone along the trail to Deer Cave at Mulu National Park, Long Lama, Marudi, Miri Division, Sarawak, Malaysian Borneo (04° 02' 02.0" N, 114° 49' 00.0" E, 40 m elevation). The population involved was ca. 100 individuals.

Fieldwork was undertaken to determine the flowering event(s) for *A. hewittii* and *A. eburneus* at least once a month between December 2015 and November 2016. Fieldwork for *A. julaihii* was carried out in March and again in August 2017. Inflorescences were identified and observed for at least a week prior to anthesis. Observations on flowering behaviours were carried out on an hourly basis from the start until the end of anthesis, indicated, respectively, by the opening of the spathe limb and onset of odour production (the onset of pistillate anthesis), pistillate zone drying and reduced floral odour (end of pistillate anthesis), and pollen release (the onset of staminate anthesis). The type and behavior of floral visitors were documented. The number of inflorescences investigated was: *A. hewittii* (n=8), *A. eburneus* (n=6), and *A. julaihii* (n=2). The observation on the flowering behaviours of *A. julaihii* was not carried out, as the number of inflorescences was limited. All images were taken using a Nikon D5200 digital camera. Additional inflorescences were bagged during pistillate anthesis for *A. hewittii* (n=1), *A. eburneus* (n=3), and *A. julaihii* (n=2) to capture the floral visitors. Insects were identified to the lowest possible taxonomic level (at least family level).

Fruit set was calculated by averaging the ratio of the total number of fruits per infructescence and the total number of pistillate flowers per inflorescence: *A. hewittii* (n=1), *A. eburneus* (n=4), and *A. julaihii* (n=3). All inflorescences, infructescences and insect visitors were preserved in 70% ethanol and deposited at the Sarawak Forestry Herbarium (SAR) and Sarawak Forestry Entomology Museum. Voucher information for each taxon is provided in the supplement Table S2.

RESULTS

DNA sequences

For *matK*, the sequence alignment resulted in a data matrix of 1,757 characters, of which 1,459 bp are constant, and 168 variable bp (9.56%) are parsimony-uninformative. The remaining 130 bp (7.40 %) are parsimony-informative resulting in 100 trees of 1476 steps (CI [consistency index] = 0.74; RI [retention index] = 0.84; RC [rescaled consistency index] = 0.62; HI [homoplasy index] = 0.26). For ITS, the sequence alignment resulted in a data matrix of 886 characters, of which 588 bp are constant, and 187 variable bp (21.11%) are parsimony-uninformative. The remaining 111 bp (12.53 %) are parsimony-informative resulting in 100 trees of 1476 steps (CI = 0.79; RI = 0.82; RC = 0.65; HI = 0.21). For PhyC, the sequence alignment resulted in a data matrix of 1,094 characters, of which 946 bp are constant, and 57 variable bp (5.21%) are parsimony-uninformative. The remaining 91 bp (8.32 %) are parsimony-informative resulting in 100 trees of 1476 steps (CI = 0.86; RI = 0.88; RC = 0.75; HI = 0.14).

Phylogenetic analyses

The datasets from *matK* and ITS regions are combined into a single DNA matrix, as the trees generated from the individual gene regions are congruent. The combined phylogenetic trees generated by the RAxML (Fig. 1) and Bayesian analyses are largely congruent in their topologies with the division of six groups within subgen. *Amorphophallus* (Sedayu *et al.,* 2010; Claudel *et al.*, 2017). In both RAxML and Bayesian analyses, the Paeoniifolius-Manta clade is a sister clade to the rest of the taxa belonging to subgen. *Amorphophallus*. The Pulchellus clade (BS = 85%; PP = 1.00) is supported but the Pusillus clade is separated into two clades (Pusillus clade I, BS= 50%; PP = 0.85, and Pusillus clade II, BS= 96%; PP = 1.00). *Amorphophallus ranchanensis* is a sister taxon to clade A, clade Pusillus II, and clade B (PP = 1.00).

Clade A comprises four taxa from the Philippines, *A. longispathaceus* Engl. & Gehrm., *A. declinatus* Hett., *A. palawanensis* Bogner & Hett., and *A. salmoneus* Hett. The species from the Philippines is closely related to *A. hottae*, a species from Sabah. *Amorphophallus julaihii* is grouped together with *A. costatus* and two novel species from Lambir National Park (North Sarawak) and Puncak Borneo (West Sarawak). Three limestone obligates, *A. brachyphyllus*, *A. eburneus*, and *A. niahensis* are supported in the same clade with *A. infundibuliformis* (BS: 73%; PP = 0.90). Clade B represented taxa which are closely related to *A. hewittii* (BS= 70%; PP = 1.00). *Amorphophallus hewittii* is not supported as a single entity. *Amorphophallus hewittii* from Ranchan (West Sarawak, on basalts) is positioned close to *A. gigas* from Sumatera, *A. plicatus* from Sulawesi, and Philippines' *A. urceolatus* Hett., A. Galloway & Medecilo. The accessions of *A. hewittii* from the rest of western Sarawak are probably *A. hewittii* as the type was described from Pangkalan Kuap. The accession of *A. hewittii* from Mulu

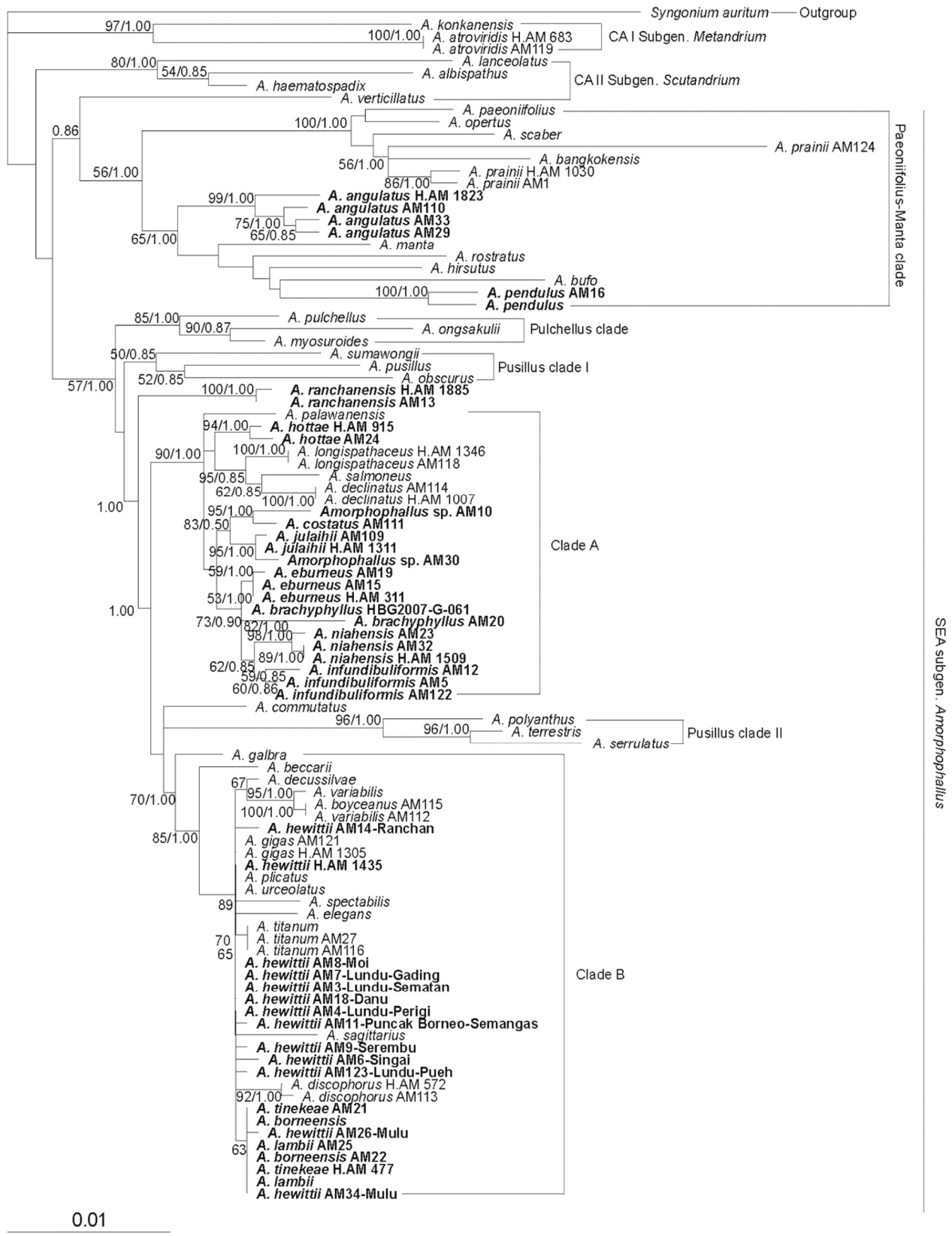

Fig. 1. Molecular phylogeny of the Bornean ***Amorphophallus*** species in the combined *matK* and ITS analysis. Bootstrap numbers from maximum likelihood (above 50%) and Posterior Probabilities (above 0.5) are provided on the branch. Bornean taxa are in bold.

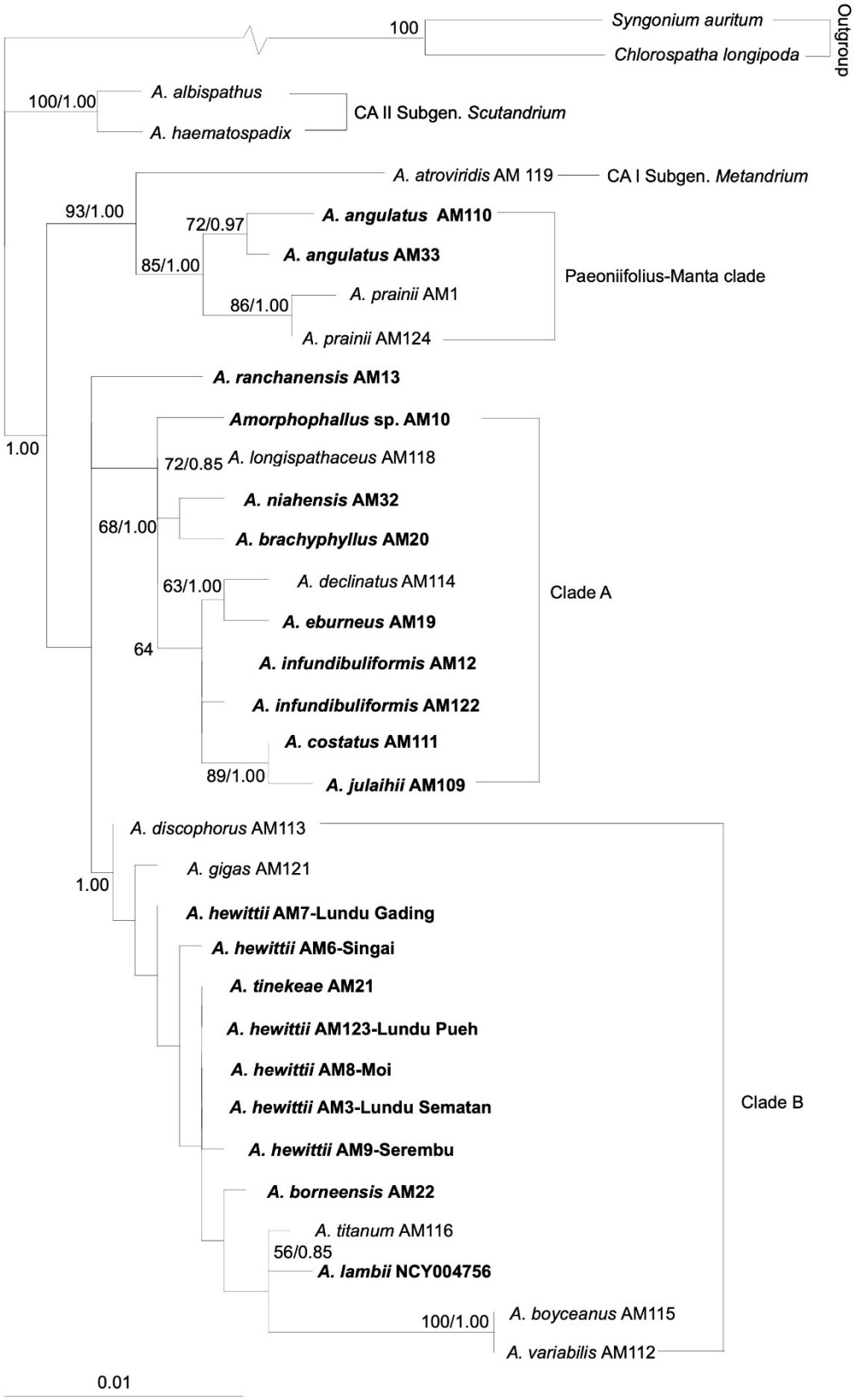

Fig. 2. Molecular phylogeny of the Bornean ***Amorphophallus*** species in the PhyC analysis. Bootstrap numbers from maximum likelihood (above 50%) and Posterior Probabilities (above 0.5) are provided on the branch. Bornean taxa are in bold.

National Park (North Sarawak, Karst-associated) is closer to the taxa from Sabah and Kalimantan (*A. tinekeae*, *A. borneensis*, and *A. lambii*) (BS = 63%) than to the West Sarawak taxa.

The relationship inferred from PhyC region (Fig. 2) reveals that the Paeoniifolius Manta-clade is linked with subgen. *Metandrium* (Claudel *et al.*, 2017) in RAxML and Bayesian analyses (BS = 93%; PP = 1.00). Both clade A and B comprised the same taxa as in the analyses of combined ITS and *matK* regions. *Amorphophallus ranchanensis* is sister to clade A. *Amorphophallus julaihii* is linked to *A. costatus* with strong supports (BS = 89%; PP = 1.00), and together they are close to *A. infundibuliformis*, *A. eburneus*, and *A. declinatus* (BS = 64%). Despite being highly similar in appearance *Amorphophallus eburneus* is not supported next to *A. brachyphyllus*, but instead *A. eburneus* is closer to *A. declinatus* (BS = 63%; PP = 1.00), with *A. brachyphyllus* next to *A. niahensis* (BS = 68%; PP = 1.00). Among taxa in clade B, *A. discophorus, A. gigas, A. tinekeae*, and *A. borneensis* are positioned together with several accessions of *A. hewittii*. *Amorphophallus titanum* is supported as a sister taxon to *A. lambii* (BS 56%; PP = 0.85), this together is related to *A. variabilis* and *A. boyceanus* (BS = 100%; PP = 1.00).

Floral biology and insect visitors

The flowering period of *A. hewittii* occurs from March until May and August until October, corresponding to the periods of lower rainfall (350–850 mm monthly; Meteorology Department Malaysia, 2016). By comparison, the flowering period of *A. eburneus* and *A. julaihii* occurs between August and October. When populations of *A. hewittii* and *A. eburneus* are found close to each other, individuals belonging to the former always flowered more profusely than those of the latter at any one time. The total numbers of mature inflorescences observed within a 10-day span each time (between 27th August and 20th October 2016) during peak season among the studied populations are as follows: *A. hewittii* (ca. 8 inflorescences of 25 individual plants) and *A. eburneus* (ca. 6 inflorescences of 300 individual plants).

Anthesis lasts ca. 49 hours in *A. hewittii* and ca. 64 hours in *A. eburneus*. The spathe of *A. hewittii* starts to open a few days before anthesis but no smell is detected. However, the inflorescences are accessible to insect visitors. The pistillate phase of anthesis for *A. hewittii* starts at ca. 11:00 am (Day 1). The spathe limb opens wide with an inflated lower spathe to allow access to the receptive pistils. Concomitantly, an intense rotting meat-like odour emission which originates from the appendix (moisture droplets are present on its surface) is detected. By 11:00 pm, Day 2, the stigmas are dry and the floral scent is no longer detected. At the same time, the staminate phase of anthesis then begins by extruding yellow pollen strings and ends by ca. 12:00 pm (Day 3). The anthesis of *A. eburneus* starts with the spathe opening at 6:00 am (Day 1) and the release of a fishy-like smell which decreases gradually. The staminate anthesis starts 24 hours later, ca. 6:00 am (Day 2) with the release of white-to-yellow pollen strings, and ends by 10 pm (Day 3). The pistillate anthesis is much longer in *A. hewitti* (36 hours) than in *A. eburneus* (24 hours) but the staminate anthesis is much shorter in *A. hewittii* (13 hours) than in *A. eburneus* (40 hours). The spathe and the spadix (excluding the pistillate zone) of *A. hewittii* wilt after anthesis, whereas for *A. eburneus*, the spathe wilts after anthesis but the upper spadix remains erect for up to several days.

A day before anthesis, several types of insects are seen within and around the inflorescences of *A. hewittii*. Several individuals of Hymenoptera (unidentified family, Fig. 3I) are found around 10:30 am and later on, individuals of Diptera (Muscidae, Fig. 3H; Drosophilidae, Fig. 3K), Hemiptera (Reduviidae, Fig. 3N), and Caelifera (unidentified family, Fig. 3P) approach the inflorescence (land on the surface of spathe and appendix). Several *Tetragonula melanocephala* (Hymenoptera: Apidae, Fig. 3J) and Muscidae (Fig. 3L) are found in the pistillate zone. Many individuals of Muscidae (Fig. 3L) are moving in and out of the inflorescences from 10:00 am (Day 1). Around 10:00 am to 11:00 am, two individuals of Silphidae beetles (Fig. 3G) and Cerambycidae beetles (Fig. 3M) are found at the base of the pistillate zone and try to crawl onto the pistils. At around 12:00 pm, several individuals of *T. melanocephala* enter the inflorescence (Fig. 3J). An unidentified fly (Fig. 3Q) lands on the spathe. During staminate anthesis, from 7:00 am (Day 3), several *T. melanocephala* individuals are seen with pollen carried on their hind legs but their movement are not restricted to the staminate zone but are also present on the pistillate zone. The appendix is damaged by Blattodea (Blattidae, Fig. 3E). During post anthesis, Scarabaeidae beetles (Fig. 3R) and Staphylinidae beetles (Fig. 3O) are observed on the spathe and the staminate zone respectively with *T. melanocephala* is found on the spadix. From the bagged inflorescences, we also found individuals of Hydrophilidae beetles, Lepidoptera (moth), and Formicidae (Table 1).

Staphylinidae beetles (Fig. 4F) are observed to enter the inflorescence of *A. eburneus* at ca. 11:30 am (Day 1). The beetles stay inside the lower chamber as they are unable to crawl up the spathe to escape. *Colocasiomyia* (Drosophilidae; Fig. 4D) is also found in the inflorescences. Several Diptera (Drosophilidae, Fig. 4E, Syrphidae, Fig. 4H, unidentified family, Fig. 4I), and Hemiptera (Cicadellidae, Fig. 4J) are also seen on the appendix and the spathe around 1 pm. From the bagged inflorescence, we also found an individual of Scarabaeidae beetles and up to five individuals of Hydrophilidae (Table 1). As for *A. julaihii*, the types of insect visitors are not as many as *A. hewittii* or *A. eburneus*, with only Staphylinidae

Fig. 3 ***Amorphophallus hewittii*****. A.** Individual plant. Inflorescence (**B.** pre anthesis, **C.** anthesis, **D.** preserved state). **E-R.** Types of floral visitors.

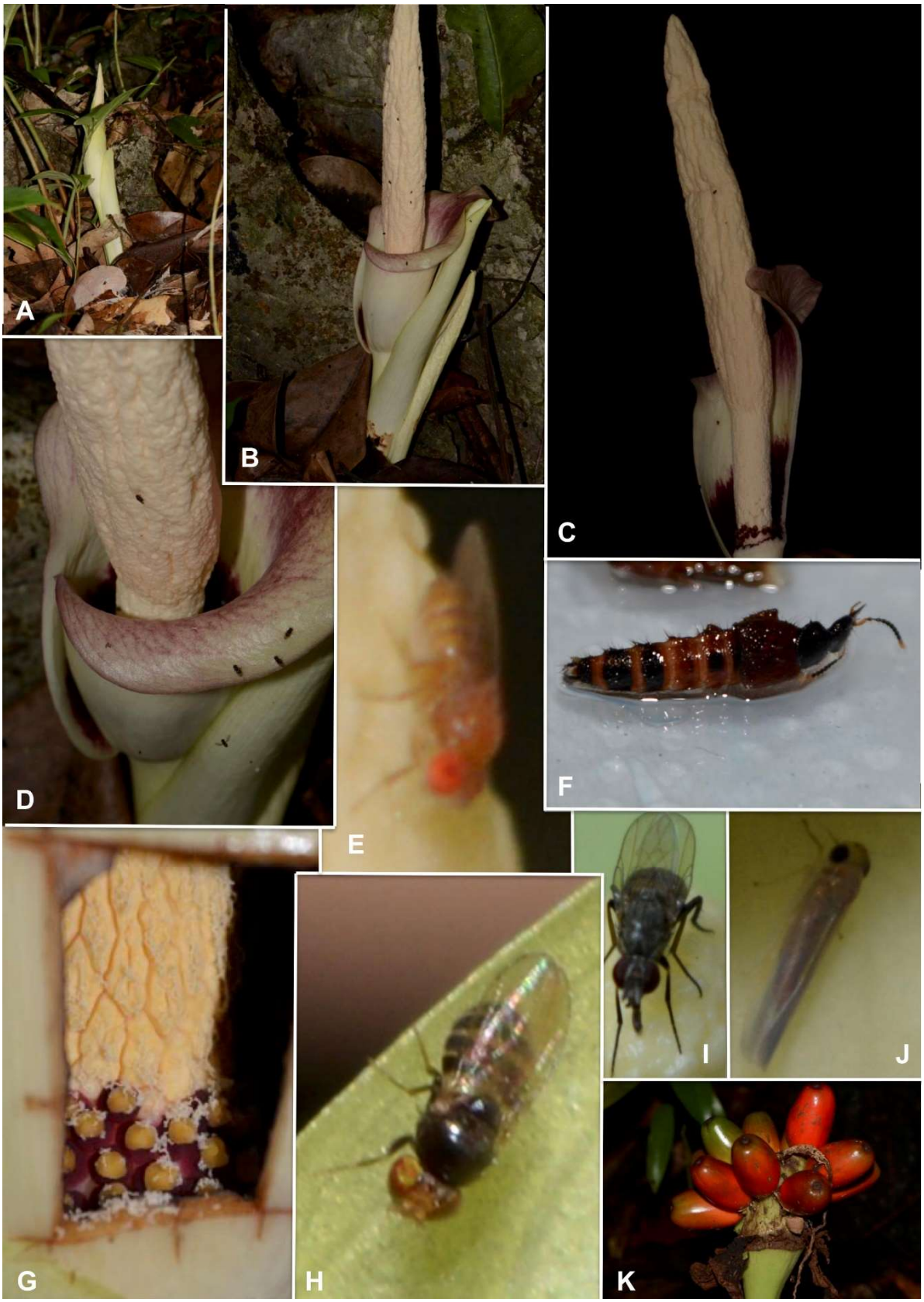

Fig. 4. ***Amorphophallus eburneus*.** Inflorescence (**A.** emerging, **B.** anthesis, **C.** spathe artificially cut off to reveal the spadix). **D-J.** Types of floral visitors. **K.** Infrutescence.

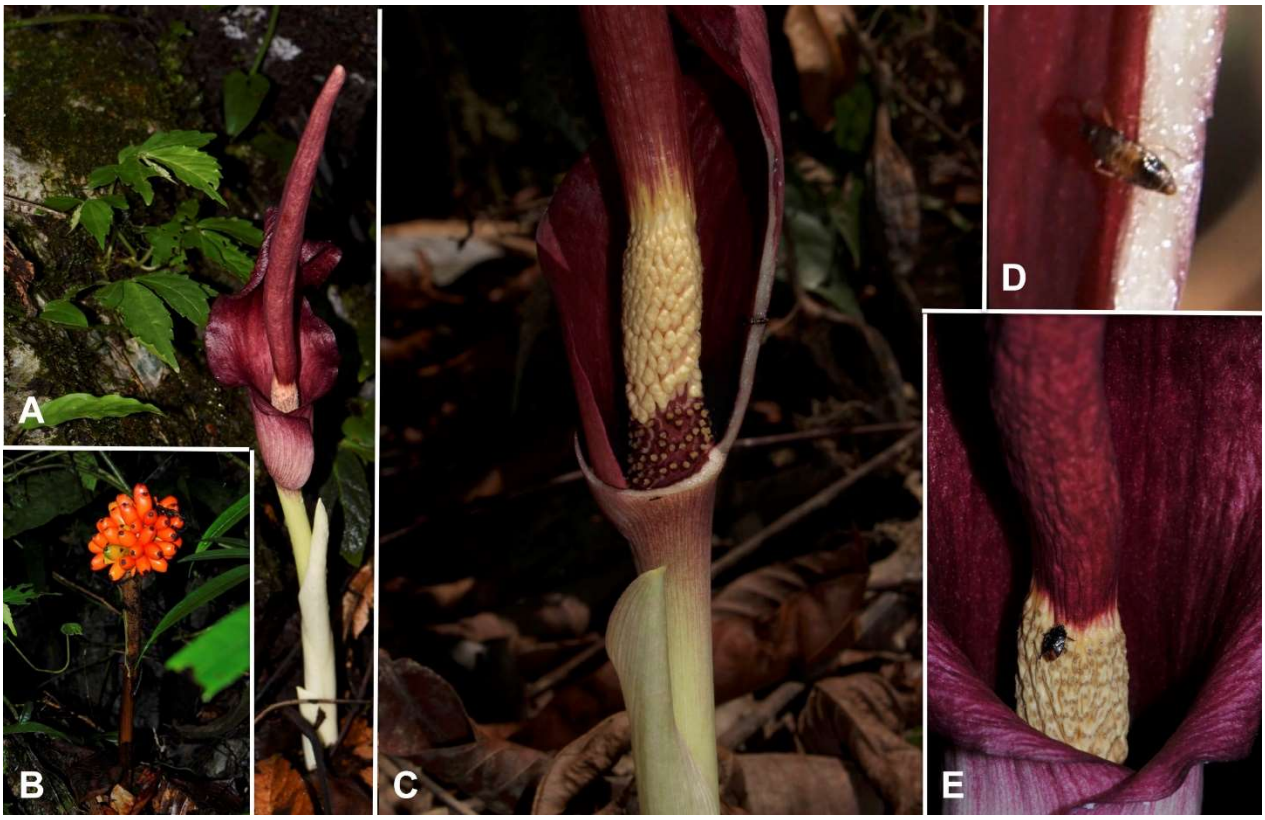

Fig. 5. *Amorphophallus julaihii*. Inflorescence (**A.** anthesis, **C.** spathe artificially cut off to reveal the spadix). **B.** Infructescence. **D & E.** Types of floral visitors.

beetles (Fig. 5D) are found during the pistillate anthesis. During staminate anthesis, in addition to the staphylinids, we observed visits from Silphidae beetles (Fig. 5E) and Thysanoptera (Table 1).

The average fruit set of *A. hewittii* is high (82.7%). Only three inflorescences out of ten inflorescences of *A. eburneus* develop into infructescences. The fruit set of *A. eburneus* and *A. julaihii* was 54.1 ± 14.4% and 75.6 ± 11.2% respectively.

Table 1. Identified species and number of insect taxa (mean±SD and range) per inflorescence of three *Amorphophallus* species.

Floral visitor	***A. hewittii***	***A. eburneus***	***A. julaihii***
Scarabaidae	2 (Fig. 3R)	1	
Staphylinidae	2 (Fig. 3O)	7-9 (3 spp.; Fig. 4F)	5-32 (Fig. 5D)
philidae	1	1-5	
Apidae	2 (Fig. 3J)		
Hymenoptera	1 (Fig. 3I)		
Blattidae	1		
Muscidae	(Fig. 3L)		
Diptera	1 (Fig. 3Q)	5-15 (*Colocasiomyia*; Fig. 4D)	
Lepidoptera	1		
Formicidae	1		
Thysanoptera			1
Silphidae	2		

DISCUSSION

Relatedness, morphology and ecology

Amorphophallus species on Borneo are separated into three clades: the Paeniifolius-Manta clade, clade A and clade B (Fig. 1). Most members of the Paeniifolius-Manta clade shared red-leafed seedling leaves and the lack of offset (small tuber) development on the tubers (Claudel *et al.,* 2017). Among the Bornean species, *A. angulatus* and *A. pendulus* belong to this clade. *Amorphophallus pendulus* was described from Matang, west Sarawak (Bogner *et al.*, 1985) and is so far not found beyond west Sarawak while *A. angulatus* which was described from Selantik, Sri Aman (Hetterscheid, 1994) occurs as far east as Brunei (third author's pers. observ.).

Clade A comprises not only all the limestone-obligated species in Sarawak, *A. julaihii*, *A. eburneus*, *A. brachyphyllus*, and *A. niahensis* but also, the non-limestone associated species, *A. hottae*, *A. costatus*, and *A. infundibuliformis*. *Amorphophallus julaihii* is closely related to *A. costatus* and both species are similar in having an erect elongate triangular spathe with the base strongly convolute forming a narrow tube, but differ by having a stipitate spadix (vs. sessile spadix in *A. costatus* and a purple spathe (vs. spathe interior glossy maroon). *Amorphophallus costatus* was however, described from Batu Besar, Datar Alei, South Kalimantan (Hetterscheid,

1994). *Amorphophallus infundibuliformis* is widespread in West Sarawak from Sematan to the Klingkang range but scattered and seldom locally abundant (Ipor *et al.*, 2004). *Amorphophallus infundibuliformis* is vegetatively strikingly different from its related limestone species but the inflorescence is closely similar with the same fishy floral odour (Kite and Hetterscheid, 1997).

Amorphophallus hewittii is found in areas adjacent to the limestones and on sandstones as well. This apparent ecological diversity may be an artefact of imperfect taxonomy. The large size of this plant, both florally and vegetatively, makes its unpopular subject for herbarium collection and thus, our knowledge of its morphology is based on a decidedly meagre collection of mediocre specimens (Ipor *et al.*, 2004). From our phylogeny, although the samples of *A. hewittii* are scattered over clade B together with 13 non-Bornean taxa and three Bornean taxa, the topologies within clade B are not well resolved. Though the subclade that includes *A. borneensis* may be monophyletic (while not strongly supported) it uncertain that the rest of *A. hewittii* samples belong to more than one clade. The non-Sarawak but related species recognized in clade B are: *A. lambii*, *A. tinekeae*, and *A. borneensis*. *Amorphophallus tinekeae* is restricted to the limestones of Gua Gomantong, Sabah. Hetterscheid and van der Ham (2001) noted that *A. tinekeae* resembles *A. borneensis*, from which *A. tinekeae* differs by having a consistently short peduncle, strongly zygomorphic stigmas and a rather narrow uninflated appendix

Floral biology and possible pollinators

Flowering behaviours of *Amorphophallus* differ among the clades. The anthesis of *A. hewittii* observed in this study differs slightly to the observations done by Chai and Wong (2019) in which anthesis started slightly later with a longer pistillate anthesis. However, staminate anthesis is similar in both investigations. Taxa belonging to this group of related taxa, *A. commutatus*, *A. titanum* and *A. variabilis*, tend to bloom in the afternoon (van der Pijl, 1937; Giordano, 1999; Punekar and Kumaran 2010). In a separate cluster in subgen. *Amorphophallus*, where *A. julaihii* and *A. eburneus* belong, the anthesis began in the morning (Chai and Wong 2019 and this study). The temporal separation between *A. hewittii* and *A. eburneus* or *A. julaihii* or possibly *A. brachyphyllus* (not studied, but co-existing with *A. hewittii*) allows allopatric co-existence in addition to inhabiting different geological and ecological aspects.

There are few studies on pollination of *Amorphophallus* species in its natural habitat (Kite *et al.*, 1998). Often for the same species, different floral visitors were reported, for example, in *A. titanum* though Giordano (1999) stated as many as 12 types of insect visitors but van der Pijl (1937) stated two possible pollinators (Silphidae and Staphylinidae). In Chai and Wong (2019), *A. hewittii* was shown to be pollinated by carrion beetles (*Diamesus*; Silphidae), dung beetles (Hybosoridae) and Staphylinidae. In this study, the possible pollinators of *A. hewittii* are: Apidae, Silphidae, Cerambycidae, and Hydrophylidae based on their presence and behaviour during both phases of anthesis. In addition to the staphylinid beetles, Chai and Wong (2019) also reported the presence of two more pollinators, Silphidae and Thysanoptera for *A. julaihii*. *Amorphophallus eburneus* and *A. julaihii* attracted fewer species of floral visitors than *A. hewittii*.

The structural diversity of the inflorescences partly reflects their adaption to the various types of pollinators (Moretto *et al.*, 2019). In this study, the morphological structures of the inflorescence in retaining the pollinators and the composition of the floral odours emitted by the spadix (Kite *et al.*, 1998, 2017) determines the type of pollinators. *Amorphophallus titanum* and *A. gigas* belonging to clade B together with *A. hewittii* are primarily pollinated by carrion beetles and dung beetles where else *A. julaihii* and *A. eburneus* (from clade A) appear to attract different pollinators (Staphylinidae and Drosophilidae). Kite *et al.* (1998) reported that *A. eburneus* and *A. brachyphyllus* emit a fishy floral odour. *Amorphophallus titanum* and *A. hewittii* were described to emit floral odour reminiscing rotting flesh and ammonia-like respectively (Giordano, 1999; Chai and Wong, 2019). Although there are no available data on the floral compounds of *A. hewittii*, in related species, for example, *A. titanum*, *A. commutatus* and *A. variabilis*, the dominant compound is dimethyl oligosulphides. Trimethylamine was the dominant component of the strongly fishy odour of *A. eburneus* and *A. brachyphyllus* (Kite and Hetterscheid, 1997, 2017), and these are well-supported sister species. So within the Bornean taxa, we could see two clusters with different major odour compounds, attracting different sets of pollinators (more types of floral visitors in dimethyl oligosulphides dominant taxa vs. less types in trimethylamine dominant taxa) though investigations on more species are needed.

CONCLUSIONS

Bornean *Amorphophallus* taxa are separated into three clades which are each morphologically well defined. This study also presents observational data on floral biology and possible pollinators of *A. hewittii*, *A. eburneus*, and *A. julaihii*; the latter two species belong to a clade where none of the species were so investigated before. Given the diversity of *Amorphophallus* on Borneo, it would be interesting to investigate further on the floral biology of particularly, *A. pendulus* and *A. angulatus* of the Paeoniifolius-Manta clade.

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