



Faculty of Resource Science and Technology

Phylogenetic Analyses and Floral Biology of *Tacca* (Taccaceae) on Borneo

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Phylogenetic Analyses and Floral Biology of *Tacca* (Taccaceae) on Borneo

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DECLARATION

The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Genus *Tacca* (Taccaceae) comprises about 20 species with pantropical distribution but mostly occurring in the Old World tropics. Most *Tacca* species were characterized by its inflorescence with conspicuous bracts and filiform bracteoles. There were two main objectives in this study, which were to resolve the phylogenetic relationship of genus *Tacca*; and to investigate floral biology of *Tacca* spp. in Sarawak based on observations, field manipulation, and controlled pollination tests. Phylogenetic relationship between *Tacca* spp. was analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) based on DNA sequences of nuclear internal transcribed spacer (ITS) and plastid maturase K (matK) region. The phylogenetic trees resulted from combined regions showed a congruent topology with work. *Tacca bibracteata* was closely related to *T. palmata*, *T. palmatifida* and *T. plantaginea*. *Tacca borneensis* formed sister clade to *T. cristata*, *T. laevis*, *T. reducta* and *T. sumatrana*. The position of *T. reducta* in the phylogenetic analysis questioned the previous inflorescence traits evolutionary hypothesis which stated *Tacca* evolved from reduced to conspicuous floral features. Mapping of morphological characters on the phylogenetic tree indicated that ancestral states of *Tacca* were palmate leaf blades, long peduncle, large outer involucre bracts, small but greater amount of inner involucre bracts, long bracteoles and higher amount of flowers with less number of ovules. For floral biology, observations were made on *T. borneensis*, *T. cristata*, *T. laevis*, *T. leontopetaloides*, *T. palmata* and *T. reducta*. Flowering period of each species varied with *T. leontopetaloides* bloomed annually; *T. borneensis* and *T. palmata* bloomed sporadically; and *T. cristata*, *T. laevis* and *T. reducta* bloomed continuously. Anthetic period began at 05:30 to 07:00 (UTC + 0800), except *T. leontopetaloides* which bloomed at 17:00. Their anthetic period lasted for

8 – 14 hours depending on species. The flowers are protogynous. Only flowers of *T. laevis* and *T. leontopetaloides* emitted faint floral scent. Pollen: ovule (P:O) ratios fall within the range of facultative self-pollinated species. In Borneo, inflorescence of *Tacca* spp. received a variety of random insect visitations with female ceratopogonid midge (*Culicoides hinnoi* & *Forcipomyia* sp.), stingless bee (*Trigona* sp.), ant (Formicidae), and mosquito (Culicidae) frequently observed. Among these insects, midge was possibly the main pollinator owing to its accessibility onto the anther and stigma, and its visitation during anthesis. Yet, the efficiency of midge in aiding to outcross *Tacca*'s flower was not clear owing to its inconsistent visitations and movement within the flower not observed. Moreover, the pollination syndrome developed by *Tacca*'s inflorescence in attracting pollinator remained unknown, so did the main purpose of developing bracts and bracteoles. Only *T. borneensis* did not produce any fruit when the floral structures removed. Pollination experiment indicated *Tacca* spp. in Borneo are self-compatible but not autonomous self-pollinated. *Tacca borneensis* showed immature fruits abortion in selfing test, indicating inbreeding depression.

Keywords: Female *Forcipomyia* sp., insect visitors, ITS, matK, pollen:ovule ratios, pollination

Analisis Filogenetik dan Biologi Pembungaan Tacca (Taccaceae) di Borneo

ABSTRAK

Genus Tacca (Taccaceae) terdiri daripada kira-kira 20 spesies yang wujud dalam lingkungan pantropis tetapi kebanyakannya berada di Old World tropika. Kebanyakan spesies Tacca dicirikan oleh perbungaannya yang mempunyai braktea yang besar dan braktea berbentuk filamen. Kajian ini mempunyai dua objektif utama, iaitu untuk menyelesaikan hubungan filogenetik genus Tacca; dan untuk mengkaji biologi pembungaan spesies-spesies Tacca di Sarawak berdasarkan pemerhatian, manipulasi di lapangan dan ujian polinasi terkawal. Hubungan filogenetik antara spesies-spesies Tacca dianalisis dengan menggunakan parsimony maksimum (MP), kemungkinan maksimum (ML) dan kesimpulan Bayesian (BI) berdasarkan urutan DNA internal transcribed spacer (ITS) dan maturase K (*matK*). Pohon filogenetik yang dihasilkan daripada gabungan urutan kedua-dua gen ini menunjukkan topologi yang sama dengan penyelidikan yang dilaporkan sebelum ini. Tacca bibracteata berkait rapat dengan T. palmata, T. palmatifida dan T. plantaginea. Tacca borneensis membentuk klad percabangan dengan T. cristata, T. laevis, T. reducta dan T. sumatrana. Kedudukan T. reducta dalam analisis ini mempersoalkan hipotesis evolusi ciri-ciri bunga majmuk sebelumnya yang menyatakan ciri-ciri perbungaan Tacca berevolusi dari kelihatan kecil kepada yang menonjol. Pemetaan karakter-karakter morfologi atas pohon filogenetik menunjukkan bahawa keadaan leluhur Tacca adalah bilah daun palma, batang bunga panjang, braktea luar yang besar, braktea dalam yang kecil tetapi lebih banyak, braktea filamen panjang dan jumlah bunga yang lebih banyak serta jumlah ovul yang kurang. Untuk biologi bunga, pemerhatian dilakukan terhadap T. borneensis, T.

cristata, T. laevis, T. leontopetaloides, T. palmata dan T. reducta. Tempoh pembungaan setiap spesies berbeza dengan T. leontopetaloides berkembang setiap tahun; T. borneensis dan T. palmata mekar secara sekali-sekala; dan T. cristata, T. laevis dan T. reducta mekar secara berterusan. Tempoh antetik bermula pada jam 05:30 – 07:00 (UTC + 0800), kecuali T. leontopetaloides yang berkembang pada jam 17:00. Tempoh antetik mereka berlangsung selama 8 – 14 jam bergantung kepada spesies. Bunga-bunga tersebut adalah jenis “protogynous”. Hanya bunga T. laevis dan T. leontopetaloides yang mengeluarkan aroma yang susah untuk dikesan. Nisbah debunga : ovul (P:O) berada dalam lingkungan spesies pendebungaan sendiri fakultatif. Di Borneo, perbungaan spesies-spesies Tacca menerima pelbagai kunjungan serangga secara rambang dengan ceratopogonid sera betina (Culicoides hinnoi & Forcipomyia sp.), kelulut (Trigona sp.), semut (Formicidae), dan nyamuk (Culicidae) sering diperhatikan. Antaranya, sera mungkin adalah pendebunga utama disebabkan aksesibilitinya ke atas anter dan stigma, dan lawatannya semasa anthesis. Tetapi, kecekapan sera dalam membantu pendebungaan kacuk Tacca adalah tidak jelas kerana lawatannya yang kurang konsisten, serta pergerakannya di dalam bunga yang tidak dapat diperhatikan. Selain itu, sindrom pendebungaan yang dihasilkan oleh bunga Tacca untuk menarik perhatian pendebunga masih tidak diketahui, begitu juga dengan tujuan utama mempunyai braktea dan braktea filamen. Hanya T. borneensis yang tidak berbuah selepas aksesori bunga dibuang. Eksperimen pendebungaan menunjukkan Tacca di Borneo adalah serasi dengan diri sendiri tetapi tidak dapat pendebungaan sendiri secara autonomi. Tacca borneensis menghadapi pengguguran buah yang belum matang dalam ujian pendebungaan sendiri, menunjukkan kemurungan pembiakbakaan dalam.

Kata kunci: Forcipomyia sp. betina, pelawat serangga, ITS, matK, nisbah debunga:ovul, pendebungaan

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LIST OF ABBREVIATIONS

AIC	Akaike information criterion
ANOVA	analysis of variance
APG	Angiosperm Phylogeny Group
atpA	adenosine triphosphate synthase subunit alpha
BI	Bayesian inference
BS	bootstrap
bp	basepair
°C	degree Celsius
cm	centimetre
CI	consistency index
CV	coefficient of variance
df	degree of freedom
diam	diameter
dNTP	deoxyribonucleotide triphosphate
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
FRIM	Forest Research Institute Malaysia
g	gram
GPS	global positioning system
HI	homoplasy index
HSD	Honest Significant Difference
ITS	internal transcribed spacer
KNP	Kubah National Park

KPL	Kampung Peninjau Lama
KS	Kampung Segong
Ma	mega-annum
m a.s.l.	metres above sea level
matK	maturase K
MCMC	Markov Chain Monte Carlo
MgCl ₂	magnesium chloride
min	minute
ML	maximum likelihood
mm	millimetre
mM	millimolar
MP	maximum parsimony
MS	Mount Singai
N/A	not available
nad1 b-c	b/c of subunit 1 of nicotinamide adenine dinucleotide dehydrogenase
no.	number
PCR	polymerase chain reaction
P:O	Pollen: Ovule
PP	posterior probability
PS	Pantai Sampadi
μL	microlitre
mM	milli mol
mm	millimetre
rbcL	large subunit of the ribulose-bisphosphate carboxylase

rDNA	ribosomal deoxyribonucleic acid
RI	retention index
RRP	Ranchan Recreational Park
sp./spp.	species
TAE	tris-acetate-EDTA
TBR	Tree bisection reconnection
V	volt

CHAPTER 1

INTRODUCTION

1.1 Research Background

Tacca J.R. Forst & G. Forst belongs to family Taccaceae (Stevens, 2001 onwards). *Tacca* species grow as acaulescent, perennial herbaceous plant in forest ground (Drenth, 1976). To date, about 20 species were described (Drenth, 1976). These species occur at the pantropical region with almost all from the Old World Tropics, except *T. parkeri* which is only recorded in New World.

Species in this genus are commonly known as bat lily and arrowroot (Zhang et al., 2007). These local names refer to the inflorescence's bracts of certain species (e.g. *T. chantrieri*) which appear like a bat. *Tacca* spp. are also known as tiger's whisker owing to the long filiform bracteoles on the inflorescence of most *Tacca* spp. (Zhang et al., 2007). These conspicuous bracts, bracteoles and flowers in *Tacca* spp. are dull, dark purple to green in colour. This characteristic is suspected to be related to sapromyophily (Zhang et al., 2005). Sapromyophily is a type of plant adaptation which some plant parts, normally inflorescences or flowers, resemble dead organic matters by emitting odour or/and colour of rotten meat to attract flies for pollination purpose (Johnson & Jurgens, 2010). Although the colour of *Tacca* spp. matches this plant behaviour, the decaying smell is absent (Zhang et al., 2005).

Despite its interesting floral structures, molecular samples of *Tacca* spp. were included as outgroups to solve phylogeny of several families within Dioscoreales (Caddick et al., 2002; Couto et al., 2017; Merckx et al., 2006; Wilkin et al., 2005). The most recent ancestral reconstruction among *Tacca* spp. concluded the evolutionary development of bracts and

bracteoles modified from inconspicuous to prominent structures (Zhang et al., 2011). *Tacca leontopetaloides*, *T. palmata* and *T. palmatifida* formed the basal of phylogeny tree, whereas *T. chantrieri*, *T. integrifolia* and *T. subflabellata* joined together within the derived clade. But, will this statement stay true? When species with even larger bracts (*T. borneensis*) or smaller bracts (*T. bibracteata* & *T. reducta*) added to the phylogenetic analysis. In addition, the phylogenetic trees indicated that *T. cristata* and *T. integrifolia* were not genetically close to each other, even though both species had similar floral morphologies (Zhang et al., 2011).

In general, studies of floral biology include not only the morphologies of flower or inflorescence but also the flowering behaviour of a plant species (Blochtein et al., 2014; Hoe & Wong, 2016). For instances, the period of anthesis, pollination syndrome and emission of fragrance are essential criteria to be investigated for the reproductive biology of a species. This information is important as plant reproduction mechanism is closely connected to the survival capability of the respective plant (Gan et al., 2013). Removal of either one of these elements may hinder the success of a flower to develop fruit. It may not show any significant problem in a short period of time, but long term removal can result in endangering the species (Gan et al., 2013).

Insect visitors are reported to be useful and effective pollinators as their population is extremely huge. Their importance in performing cross-pollination for flowers was observed in most flowering plant including wild herbs, cash crops, and tree species (Tepedino, 1979). Insect pollinators such as bees, butterflies, beetles, flies and other insects adapt as mutual beneficial partners to plants. For instances, plant provides reward such as nectar and breeding site, in return of insect aiding in distribution of pollen to another plant (Sakai, 2002). Moreover, special landing platforms, flower colouration, spurs and nectaries existed in some orchids to attract specific pollinator group such as beetles and wasps (Johnson et al., 2007).

Generally, flowers comprised of petals and sepals for attracting pollinators and protection purposes. In some families such as Araceae and Zingiberaceae, petals and sepals usually are reduced but extrafloral organs are developed to replace the function of corolla and calyx. There are lots of variable bract types. Some species have large, colourful bracts (e.g. *Bougainvillea* spp.), while others have green, inconspicuous bracts (e.g. maize). Different types of bracts play specific role in aiding reproductive mechanism of species. For instances, some bracts are important in attracting pollinators (e.g. sunflower, and *Euphorbia* spp.; Gagliardi et al., 2016). Bracts are sometimes useful in ensuring effective fertilization by protecting the reproductive parts from inconsistent environment conditions (Sun et al., 2008; Yang & Sun, 2009). Bracteoles were also recorded to provide nectar reward and protect their pollinators in genus *Macaranga* (Yamasaki et al., 2015).

For genus *Tacca*, bracts and bracteoles are apparently obvious, but their functions are still not known (Zhang et al., 2005; Zhang et al., 2007). The experiment of removing bracts and staking the inflorescence of *T. chantrieri* did not give any evidence on the effects of bracts on pollination success. In addition, no effective pollinator was recorded and *T. chantrieri* was reported as autonomous self-pollinating species (Zhang et al., 2005). In another *Tacca* sp., however, female Ceratopogonid midges were observed visited flowers of *T. cristata* (Lim & Raguso, 2017). Ceratopogonid midges were strongly believed to aid the outcrossing of *T. cristata* as they were observed visited the anthetic flowers with pollen (Lim & Raguso, 2017). The differences in findings suggested there is variation in pollination strategies adopted by different *Tacca* spp.

1.2 Problem statements

The main research gap of current phylogenetic on *Tacca* spp. was that all *Tacca* spp. from Borneo were not included in the phylogenetic analysis conducted by Zhang et al. (2011). Thus, the relationship of *Tacca* spp. from Borneo with other species within the genus remained unknown. There were only limited publications on the floral biology of *Tacca* spp. *Tacca chantrieri* and several other species were reported to perform autonomous self-pollination (Zhang et al., 2011). However, *T. cristata* was reported to have female Ceratopogonid midge visitation, suggesting cross-pollination might exist in genus *Tacca* (Lim & Raguso, 2017). Nevertheless, no floral biology study of *Tacca* spp. was conducted in Sarawak.

1.3 Objectives

In this project, the objectives were stated as the following:

- a. To resolve the phylogenetic relationship among *Tacca* spp. on Borneo within the genus.
- b. To investigate the floral biology and pollination strategies of selected *Tacca* spp.
- c. To investigate the role of conspicuous floral structures with pollination success of selected *Tacca* spp.
- d. To determine the possibility of self- or cross-pollination of selected *Tacca* spp. through artificial pollination tests.

CHAPTER 2

LITERATURE REVIEW

2.1 *Tacca* J.R. Forst. & G. Forst.

2.1.1 Taxonomic history

Tacca J.R. Forst. & G. Forst. belongs to the family Taccaceae as the type genus (Drenth, 1972). The genus *Tacca* was established with the type species, *Tacca pinnatifida* J.R. Frost & G. Frost in the year 1775. Despite the fact that *T. pinnatifida* was the type species, *Leontice leontopetaloides* L. was the first named species of the genus with valid publication. In 1891, O. Kuntze transferred *Leontice leontopetaloides* into genus *Tacca*, with the name *T. leontopetaloides* (L.) Kuntze. By that time, the genus *Tacca* was conserved because it had an overall 30 accepted species. This conversion rendered *T. pinnatifida* a synonym of *T. leontopetaloides* (Drenth, 1972).

Taccaceae used to be comprised of two genera, in which the other genus is *Schizocapsa* Hance which had *Schizocapsa plantaginea* Hance as the type species. *Schizocapsa* was delimited by their fruit with capsules which dehisce to disperse seeds when ripening (Ding & Larsen, 2000). However, other distinctive characteristics of *Schizocapsa* such as smaller sized leaf lamina and anomocytic arranged stomata were also observed in some *Tacca* spp. (e.g. *T. reducta* with leaf blade size 23 – 30 x 5 – 9 cm, and *T. palmata* with stomatal apparatus anomocytic type) (Boyce & Julia, 2006; Flora Reipublicae Popularis Sinicae, 2004). Moreover, genus *Schizocapsa* had only two recognized species, namely *S. plantaginea* and *S. guangxiensis*. However, *Schizocapsa* is now treated as a synonym of *Tacca*, with its constituent two species treated as synonyms of *T. plantaginea*.

In Drenth (1972), species in genus *Tacca* were reduced to 10 accepted species (*T. leontopetaloides*, *T. integrifolia* Ker Gawl., *T. plantaginea* (Hance) Drenth, *T. chantrieri* Andre, *T. bibracteata* Drenth, *T. palmata* Blume, *T. ebeltajae* Drenth, *T. palmatifida* Baker, *T. celebica* Koord., and *T. parkeri* Seem.). Although *T. maculata* Seem. was regarded as a synonym of *T. leontopetaloides* by Drenth (1972), later surveys still recognized it as an accepted species (Cowie & Stuckey, 2008; Keighery et al., 1995). *Tacca subflabellata* P.P. Ling & C.T. Ting was later discovered in Yunnan, China (Ling & Ting, 1982). In 1997, *Tacca ankaranensis* Bardot-Vaucoulon was described as a novel species in Madagascar (Bardot-Vaucoulon, 1997). It is distinctive with palmatilobate leaves and only two filiform bracteoles. Previously, *T. borneensis* Ridl. in Borneo was recognized as one of the synonyms of *T. integrifolia*. It was later resurrected owing to its distinctly different floral structures from *T. integrifolia* (Boyce & Julia, 2006). One of the obvious characteristics which differentiate the two species is the inflorescence bracts which are heteromorphic in *T. integrifolia*, but homeomorphic in *T. borneensis*. At the same time, a new species, *T. reducta* P.C. Boyce & S. Julia was described in Borneo. In 2008, another species, *T. amplipecta* L. Zhang & Q.-J. Li, at Yunnan, China was found. This species had vegetative parts resemble *T. chantrieri*, and it was suggested to be the hybrid produced by *T. chantrieri* and *T. integrifolia* (Zhang & Li, 2008a). Another new species was discovered at Vietnam with the name *T. khamhaensis* V.S. Dang & Vuong. Inflorescence of this species was similar to *T. amplipecta* and *T. chantrieri* (Dang et al., 2018).

Currently, there are a total of 17 species with valid publication in this genus. Nevertheless, several synonymised epithets under *T. integrifolia* are in need of revision. According to Zhang et al. (2011), phylogenetic analysis on genus *Tacca* discovered that *T. integrifolia* in South East Asia did not group with *T. integrifolia* from Tibet. *Tacca integrifolia* was

originally described from eastern India. Thus, *T. cristata* Jack (*T. integrifolia* in Peninsular Malaysia and Singapore) and *T. laevis* Roxb. (*T. integrifolia* in Borneo) are possibly valid species.

The family Taccaceae together with Trichopodaceae were merged into Dioscoreaceae by Angiosperm Phylogeny Group (APG) II. It is because within the order Dioscoreales these two families had only one genus, including them under the same family would ease the taxonomic work on these families in the future. In addition, the intergeneric support value for genus *Tacca* in the phylogeny of Dioscoreales was weak (bootstrap less than 50) based on Caddick et al. (2002). In the latest APG IV, Taccaceae was maintained as a subfamily under the family Dioscoreaceae. Inflorescences of all *Tacca* spp. had some basic floral morphologies despite their differences (Figure 2.1). Figure 2.2 and 2.3 indicated the inflorescences of *Tacca* spp. for comparing the floral morphology variations among species (e.g. shape of bracts, number of bracteoles).

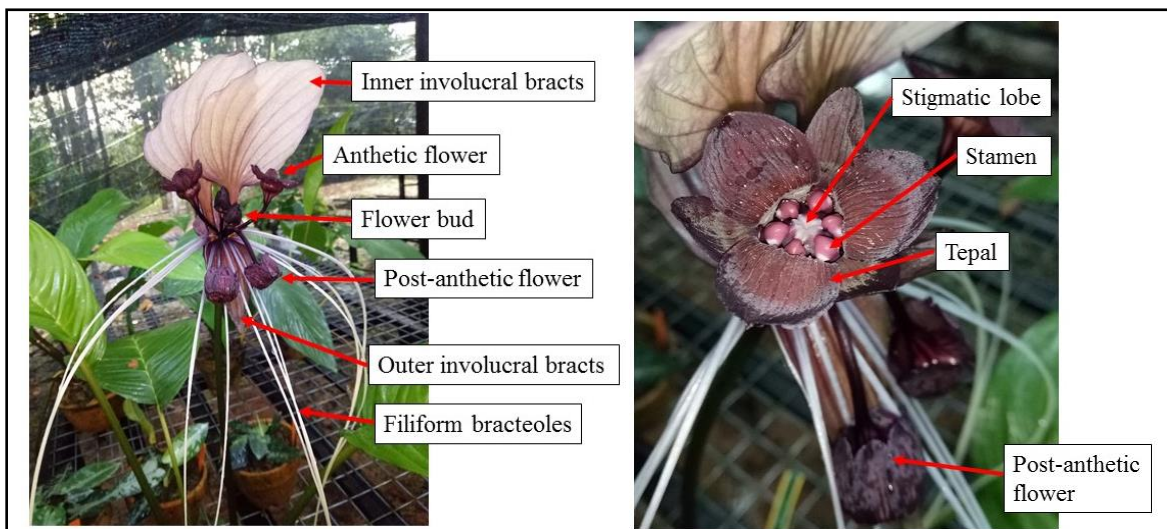


Figure 2.1: Floral morphologies of *Tacca* spp. (based on the inflorescence of *T. integrifolia*).

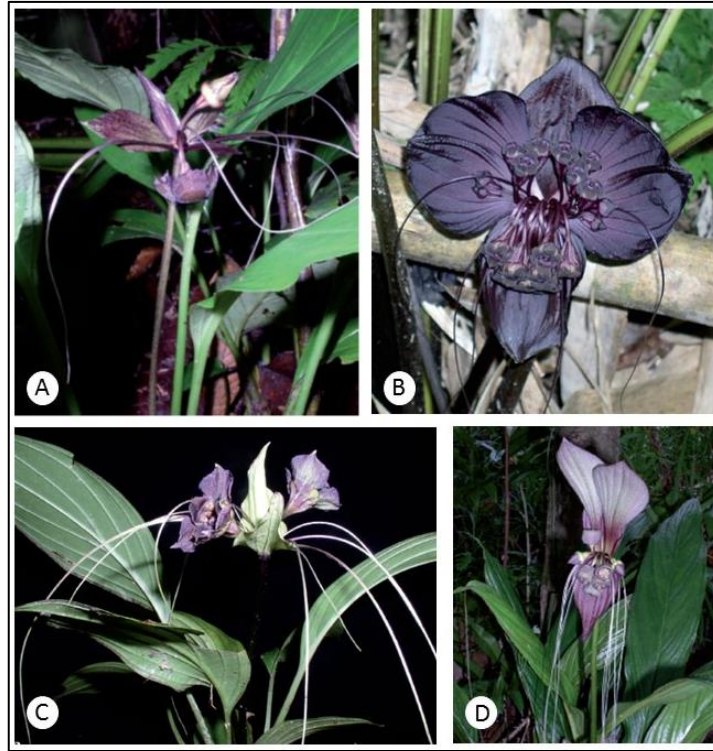


Figure 2.2: Inflorescences of *Tacca* spp. in Borneo (A: *T. reducta*, B: *T. borneensis*, C: *T. bibracteata*, D: *T. laevis*; Images retrieved from Boyce & Julia, 2006).

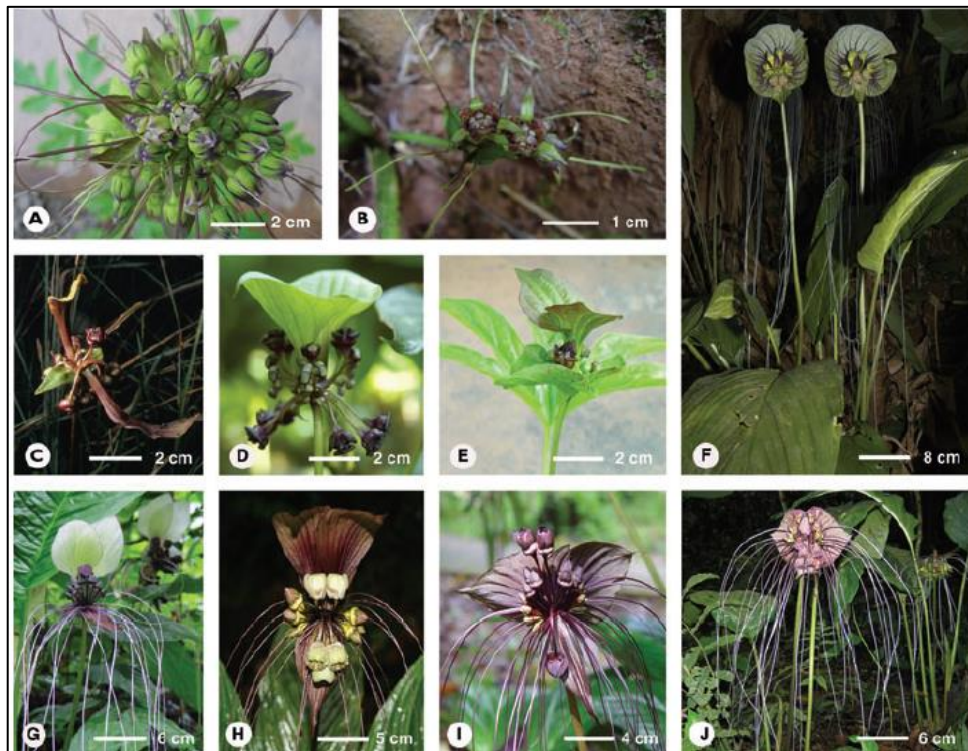


Figure 2.3: Inflorescences of *Tacca* spp. (A: *T. leontopetaloides*, B: *T. plantaginea*, C: *T. parkeri*, D: *T. palmatifida*, E: *T. palmata*, F: *T. subflabellata*, G: *T. integrifolia*, H: *T. cristata*, I: *T. amplipecta*, J: *T. chantrieri*; Image retrieved from Zhang et al., 2011).

2.1.2 Sections and groups in *Tacca*

The earliest classification of the section in *Tacca* was established by Pax in 1887, dividing species according to their sterile morphology and floral structure. Two groups were proposed namely *Ataccia* (Presl) Pax, and *Eutacca* Pax. Species under *Ataccia* had entire margin leaves with filiform bracteoles, whereas *Eutacca* had species of palmatisect leaves, pinnate venation, and filiform bracteoles. Later, Limpricht in 1928 added another group *Palmotacca* Limpr. with species of palmate leaves without filiform bracteoles. However, some species in *Palmotacca* such as *T. ulei* and *T. sprucei* were redefined as synonyms of *T. parkeri* which was placed under *Ataccia*. Moreover, *T. parkeri* did not fit into any of the section as its leaves were sometimes lobed. *Eutacca* was only having a single species, *T. leontopetaloides*. Hence, this classification was rejected by Drenth (1972).

In Drenth's classification system, four groups were formed based on morphologies of ten *Tacca* spp. (Drenth, 1972). The first group consisted of four species from the Old World region with entire leaves, filiform bracteoles, and vertical elongate rhizome with apical growth (Drenth, 1972). These four species are *T. bibracteata*, *T. chantrieri*, *T. integrifolia*, and *T. plantaginea*. The second group also had four species from the Old World region, but these species did not develop filiform bracteoles (Drenth, 1972). Species in this group can be split into species with apical growth rhizome and flowers are located between involucre bracts (*T. palmata* & *T. ebeltajae*), and species without distinct apical growth on the horizontally growing rhizome and flowers are located within the inner involucre bracts (*T. palmatifida* & *T. celebica*) (Drenth, 1972). The third group comprised of one species, *T. leontopetaloides*, which is widely spread in the Old World region (Drenth, 1972). This species is different by developing palmate leaves, filiform bracteoles, and tuber with apical growth (Drenth, 1972). The last group included a New World species, *T. parkeri*, in which

it shared morphologies with the previous first and second groups (Drenth, 1972). *Tacca parkeri* sometimes does not develop filiform bracteoles, occasionally develop only 1 or 2 filiform bracteoles (Prieto-C et al., 2000). In addition, its leaves are sometimes entire margin, and sometimes with lobes (Drenth, 1972). However, the phylogenetic result of later study did not comply with this categorization (Zhang et al., 2011; further discussion in 2.1.3 below).

Based on the rhizome characteristics, *Tacca* spp. can be divided into three groups (Drenth, 1976). The types of rhizome are vertical elongate rhizome with apical growth (e.g. *T. integrifolia*), horizontal elongate rhizome without apical growth (e.g. *T. parkeri*), and roundish rhizome with apical growth (e.g. *T. leontopetaloides*) (Drenth, 1976). According to Saw (1993), the genus can be categorized into two groups based on the fruit development forms. In the first form of fruit development, the infructescence bends at the base of fruit stalk, settling the maturing fruits on the ground (Saw, 1993). Examples of species which adopt this development form are *T. chantrieri* and *T. palmatifida* (Saw, 1993). The fruits were predicted to be dispersed by rodents (Saw, 1993). Another form has infructescence remains standing after the fruit matured (Saw, 1993). The ripe fruits have bright-coloured exocarp which was assumed to attract avian for dispersal (Saw, 1993). The species with this development form are *T. palmata* and *T. leontopetaloides*.

2.1.3 Phylogeny of genus *Tacca*

There is yet to be a complete phylogenetic study of *Tacca* to include all species described (c. 16 recognized species). The interfamilial and generic relationship of order Dioscoreales

Mart. were investigated, with several species from genus *Tacca* included (Caddick et al., 2002; Couto et al., 2017; Merckx et al., 2006; Wilkin et al., 2005).

In the phylogenetic analyses of Caddick et al. (2002), six *Tacca* spp. were added to represent the genus in the reconstruction of the order Dioscoreales. These species were *T. artocarpifolia*, *T. chantrieri*, *T. integrifolia*, *T. leontopetaloides*, *T. palmata*, and *T. plantaginea*. *Tacca artocarpifolia* is a synonym of *T. leontopetaloides*. The plastid gene regions *rbcL* and *atpB* were separately analyzed. The presented tree was randomly selected from 5000 parsimonious trees (Figure 2.4). The resulting tree topologies from both data indicated that *T. integrifolia* was located nearer to the ancestral node, whereas *T. leontopetaloides*, *T. palmata*, and *T. plantaginea* appeared in the advanced lineage (Caddick et al., 2002). These clades had an overall high supporting value.

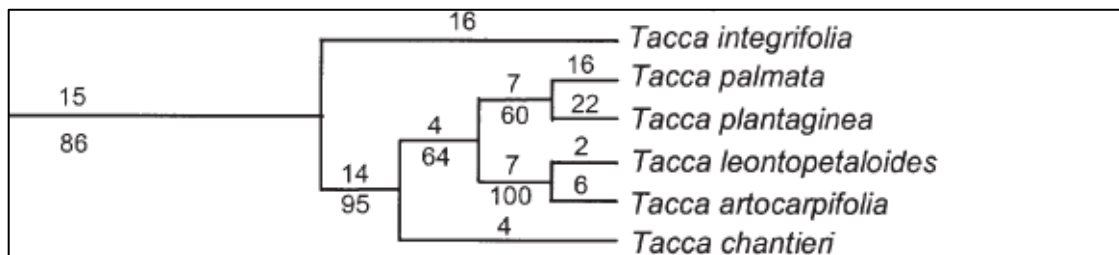


Figure 2.4: Branch lengths and bootstrap values are indicated above branches and below branches, respectively (Retrieved from Caddick et al., 2002).

Next, maximum parsimony analysis conducted by Wilkin et al. (2005) showed tree topologies incongruent to the one presented in Caddick et al. (2002). The analyzed data set was a combination of plastid *rbcL* and *matK* genes. This study focused on resolving the relationship within the genus *Dioscorea*. A similar group of *Tacca* spp. was analyzed with the addition of *T. palmatifida*. Species from genus *Trichopus* were reported to be having a close relationship with the genus *Tacca*, though clades of both the genera collapsed in the

strict consensus tree (Figure 2.5; Wilkin et al., 2005). *Tacca leontopetaloides* was sister to the rest of the *Tacca* spp. (Wilkin et al., 2005). This was followed by *T. plantaginea* (Wilkin et al., 2005). Species with palmate leaves and no bracteoles (*T. palmata* & *T. palmatifida*) joined the same subclade with high supporting value (BS = 99). Lastly, *T. chantrieri* and *T. integrifolia* formed a subclade sister to group with *T. palmata* and *T. palmatifida*.

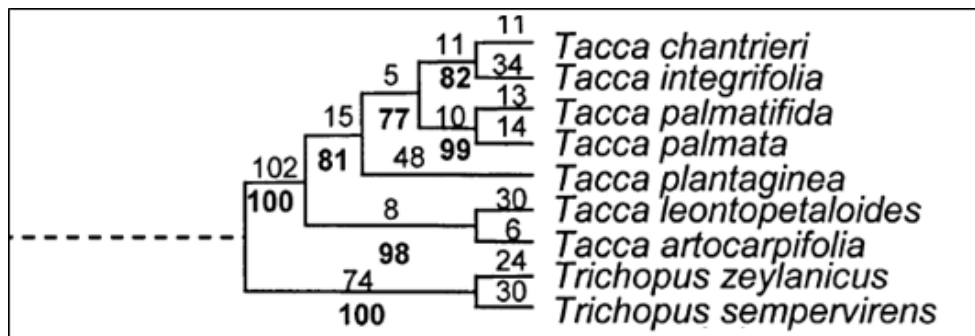


Figure 2.5: Branch lengths and bootstrap values are indicated above branches and below branches, respectively (Retrieved from Wilkin et al., 2005).

Another independent study, which involved genus *Tacca*, aimed to unravel the phylogenetic relationship of mycoheterotrophic family Burmanniaceae within the order Dioscoreales. The genus *Tacca* was found to be closely related to genus *Thismia* with the combined molecular data from nuclear 18S rDNA and mitochondrial nad1 b-c intron region (Merckx et al., 2006). This result rendered both families Dioscoreaceae and Burmanniaceae as paraphyletic. Besides the finding, the topology for *Tacca* spp. was found to be similar to result from Wilkin et al. (2005). The *Tacca* spp. included were *T. chantrieri*, *T. integrifolia*, *T. leontopetaloides* and *T. palmatifida*. Although only four species analyzed, *T. leontopetaloides* also formed the basal of the *Tacca* clade, followed by *T. palmatifida*, and then *T. chantrieri* and *T. integrifolia* (Merckx et al., 2006).

Zhang et al. (2011) analyzed the relationships of nine *Tacca* spp. and produced tree topology similar to the results of Wilkin et al. (2005) and Merckx et al. (2006) (Figure 2.6). The included species were *T. amplioplacenta*, *T. chantrieri*, *T. integrifolia*, *T. leontopetaloides*, *T. palmata*, *T. palmatifida*, *T. parkeri*, *T. plantaginea* and *T. subflabellata*. The gene regions used for phylogenetic reconstruction were amplified from nuclear genome (internal transcribed spacer), mitochondrial genome (atpA), and chloroplast genome (rbcL, trn L-F, & trn H-psbA). The combination of these five gene regions yielded tree with *T. leontopetaloides* formed the base of the monophyletic *Tacca* clade. The outgroup species in the analyses was *Trichopus sempervirens*. The only species from Neotropical region, *T. parkeri*, formed a strongly supported subclade with *T. plantaginea*. Another interesting finding from the reconstructions was the paraphyletic position of *T. integrifolia* from two different localities. *Tacca integrifolia* from Tibet, China joined the clade which comprised of *T. amplioplacenta*, *T. chantrieri* and *T. subflabellata* with locality at Yunnan, China. The other *T. integrifolia* M (*T. cristata*) from Malaysia was sister to the earlier clade. All these clades had high bootstrap support and posterior probability values (Zhang et al., 2011). However, not one Bornean *Tacca* sp. was included.

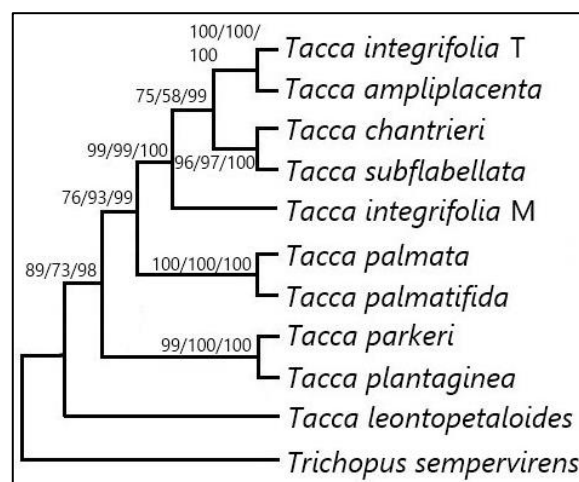


Figure 2.6: Phylogenetic relationship within *Tacca*. Numbers on branch (from left to right) are bootstraps from maximum likelihood and maximum parsimony, and posterior probability from bayesian inference (Retrieved from Zhang et al., 2011).

The ancestral inflorescence morphologies were concluded to be less showy bracts and shorter or no bracteoles (Zhang et al., 2011). Species with conspicuous floral features were located in the derived clade. This result disagrees with one of the hypotheses stated in the review of Zhang et al. (2007) in which the bracts and bracteoles lose their function as time passes. Despite of these discoveries, the assumption of the attractiveness of bracts and bracteoles toward pollinators is doubtful as other *Tacca* spp. with conspicuous floral structures was reported to be self-pollinated (Zhang et al., 2005, 2006, 2007, 2011). The literature on the floral biology of *Tacca* was further discussed in 2.3.1.

2.1.4 Hypotheses on the evolution history of genus *Tacca*

Several hypotheses of the relationship between self-pollinating ability and development of showy bracts and bracteoles had been stated by Zhang and colleagues (2007). These assumptions also explained the sapromyophilous syndrome which appeared to be acquired by *Tacca* spp. The first hypothesis proposed spatial and temporal factors which affect *Tacca*'s mating patterns (Zhang et al., 2007). It is possible that other geographical regions (other than the researched areas) can allow their floral traits effective in attracting flies for cross-pollination (Zhang et al., 2007). Another possibility is the period of high fly population density for sapromyophily to occur is limited to some months or years only (Zhang et al., 2007). When pollinator quantity is low, *Tacca* spp. will reproduce by self-pollination to maintain offspring production (Zhang et al., 2007).

The second hypothesis suggested by Zhang and colleagues (2007) was the sapromyophilous syndrome is a relict condition in which *Tacca* spp. had replaced with self-pollination. This theory supposed that ancient species of *Tacca* once attracted flies with their pronounced

bracts and probably together with rotten-meat odour for pollination purposes. However, in today's environment, the original role of bracts somewhat ceased and maybe eventually reduced in the future due to selection. It is because the floral resource investment for maintaining showy floral structure maybe diverted to other plant part growth.

The third hypothesis predicted that these exaggerated floral structures may serve other functions among *Tacca* spp. In Zhang et al. (2007), they doubted the photosynthetic involvement of bract in producing essential nutrient for fruiting due to the non-optimal vertical orientation of bracts in capturing sunlight. Function in attracting seed dispersal agents is also considered a possibility (Zhang et al., 2007). Besides that, bracts also act as protective structures in several species which have inflorescence with bracts. For example, bracts of *Saussurea velutina* W.W. Smith (Asteraceae) protect inflorescences from cold weather at higher altitude areas. According to Yang and Sun (2009), removal of bracts decreased the growth of inflorescence and reduced the seed set of *S. velutina*. Another example of species, namely *Davidia involucrata* Baill. (Nyssaceae), has developed bracts that play a role in protecting pollen from rain damage besides aiding in attracting pollinators (Sun et al., 2008).

2.2 *Tacca* on Borneo

Out of about 20 currently recognized species of genus *Tacca*, six species are found in Borneo Island. Four species of the six are endemic to Borneo (Boyce & Julia, 2006). These species are *T. bibracteata*, *T. borneensis*, *T. laevis* and *T. reducta*. *Tacca bibracteata* is narrowly geographical distributed where its occurrence is restricted in Northeast area of Sarawak state. There was no detailed report on the distribution of *T. borneensis* and *T. laevis* at Borneo

since they were both previously considered as a synonym of *T. integrifolia*. These two species were recorded spreading throughout Sarawak (Boyce & Julia, 2006). *Tacca reducta* was discovered in West Sarawak and probably in West Kalimantan as well (Boyce & Julia, 2006). The other two species are *T. leontopetaloides* and *T. palmata*. Within the Old World region, *T. leontopetaloides* was recorded to be found spreading widely across the Tropics areas (Drenth, 1972). Whereas, the distribution for *T. palmata* is scattered in South East Asia (Drenth, 1972). The morphological differences among species in Sarawak are referred in the key as followed:

Key to *Tacca* in Sarawak (Retrieved from Boyce & Julia, 2006)

- 1a. Mature leaf lamina palmate or dracontoid (elaborated forms of sagittate, hastate or trisect leaves in which the anterior and posterior divisions are highly dissected and subdivided) .. 2
- 1b. Mature leaf lamina entire 3
- 2a. Mature leaves dracontoid. Ripe fruits green, ribbed. Plants of coastal forest on almost pure sand *T. leontopetaloides*
- 2b. Mature leaves palmate. Ripe fruits red, smooth. Plants of a variety of habitats, but never in coastal forest on sand *T. palmata*
- 3a. Involucral bracts strongly heteromorphic; outer involucral bracts ovate 4
- 3b. Involucral bracts homeomorphic 5
- 4a. Inner bracts filiform and not readily distinguished from filiform floral bracts (i.e., inflorescence with the appearance of only one pair of involucral bracts). Perianth persistent until fruit maturity. Plants of shale *T. bibracteata*

- 4b. Inner bracts spatulate, ascending. Perianth marcescent early in fruit development. Plants of sandstone and (rarely) limestone *T. laevis*
- 5a. Involucral bracts broadly ovate. Leaf base rounded, oblique, truncate. Perianth marcescent early in fruit development. Plants of sandstone *T. borneensis*
- 5b. Involucral bracts linear-triangular to narrowly ovate-triangular. Leaf base acute, decurrent. Perianth persistent until fruit maturity. Plants of limestone *T. reducta*

2.3 Floral biology and reproductive process

Sustainability of a plant population health relies on the effectiveness of the plant breeding system (Barrett, 1987). Plant breeding system is divided into asexual and sexual reproduction. Asexual reproduction involves the generation of new individuals which are genetically identical to its parent (de Meeus et al., 2007). This mode of reproduction can be divided into two classes: vegetative propagation and apomixis (de Meeus et al., 2007). Vegetative reproduction utilizes only parts of sterile plant in sporophytic stage without undergoing the formation of embryos/seeds to produce new sporophytes. In the case of apomixis, seeds are produced from unfertilized ovules or somatic cells from ovary tissues (Bicknell & Koltunow, 2004). In contrast, sexual reproduction in angiosperms produces embryo through the fertilization of female gamete within ovary by the male gamete from pollen (Willemse, 2003). Under the context of this mechanism, plant can perform cross-fertilization or self-fertilization or even both simultaneously (Panawala, 2017a).

Cross-pollination is categorised into allogamy and xenogamy. Allogamy happens when pollen of a flower deposit on the stigma of another flower regardless of the same or different plant. Xenogamy requires the deposition of pollen of a flower on the stigma of another flower

of a different plant of same species. Since plant is immobile, they rely mainly on biotic vectors (some abiotic) to aid in pollen transportation (Kearns et al., 1998; Klein et al., 2007). According to Charlesworth and Charlesworth (1987), outcrossing species are always associated with attractive floral appearance. Flowering plants which adapt to cross-pollination develop strategies such as scent, mimicry, and entrapment to attract pollinators. In order to receive repeated visitation, floral rewards, either nutritive or non-nutritive, were provided (Simpson & Neff, 1981). Spatial and temporal isolations on male and female reproductive organs such as herkogamy and dichogamy are normally adopted by outcrossing species to prevent selfing (Brunet & Eckert, 1998).

Outcrossing promotes evolution as undesirable characters from ancestral generations are selected and removed in offspring generations (Panawala, 2017b). Moreover, genes are recombined during meiosis resulting in offsprings inherit with varying phenotypes (Lobo & Shaw, 2008). Although cross-pollination is considered genetically superior and improved, environmental factors such as irregular weather condition diminish the population of pollinators, thereby reduces the opportunity for outcrossing (Kowalkowska & Margonska, 2012). Yet, pollinator abundancy was doubted as the entire reason for the development shift toward self-pollination (Herrera et al., 2001). Due to the constraints, adapting from outcrossing to selfing had become a common evolutionary direction in most flowering plants (Stebbins, 1974).

Self-pollination happens when pollen grains of a flower deposited on the stigma of the same flower or different flowers of the same plant. These flowers usually do not require pollination agents, but depend on abiotic factors such as floral movement to achieve pollination. Self-pollination can be categorized into three groups: autonomy, geitonogamy, and cleistogamy. Autogamy is the pollination occurred within a single flower. Geitonogamy (also an example

of allogamy) happens between two or more flowers of the same plant. Flowers which exhibit cleistogamy perform self-pollination before blooming (Panawala, 2017a).

Autonomous self-pollinated flowers develop reproductive assurance which ensures pollination to take place when the pollinator is scarce. There are three categories of reproductive assurance mechanisms, namely “prior”, “competing” and “delayed” which happen before, during or after the timing of pollinator visitation (Lloyd & Schoen, 1992). Prior autonomous selfing system was observed in *Silene noctiflora* L. (Davis & Delph, 2005), and *Tillandsia multicaulis* Steud. (Bush, 2009). Example of competing autonomous self-pollination was *Roscoea schneideriana* (Loes.) Cowley (Zhang & Li, 2008b). Although the occurrence of self-pollination before or during anthesis ensures confirmed fruit set, effects of pollen discounting on outcrossing raises steeply (Fishman, 2000). Thus, the value of reproductive assurance reduces which lead to a highly inbred offspring generation. However, autonomous self-pollination especially prior selfing maintains species integrity by creating a reproductive isolating barrier between different species to prevent hybrid formation (Brys et al., 2016).

Among these three mechanisms, delayed autonomous self-pollination is considered as adaptive because this mode promotes outcrossing while guaranteeing pollination to occur if the pollinators are absent. Generally, this mechanism is achieved at the end of floral anthesis through the changes in stamen and carpel arrangement which allow pollen deposition on stigma without any external vector (Lloyd & Schoen, 1992). For instance, corolla abscission of *Incarvillea sinensis* Lamarck with the aid of wind pushed the anthers toward the stigma when anthesis ended (Qu et al., 2007). Another newly discovered delayed selfing mechanism is by transporting pollen with mobile secreted medium. For example, *Caulokaempferia*

coenobialis (Hance) K. Larsen used self-secreted lipids to transfer pollen from anther to stigma to achieve fertilization when no pollinator visited (Wang et al., 2004).

Although selfing eases the reproduction of plant, beneficial and deleterious characters from parent generation are both retained. Hence, plants which perform selfing tend to suffer from inbreeding depression. Inbreeding depression becomes the main cost resulting from selfing as inbreeding depression reduces offspring's vigour (Lara-Fioreze et al., 2016), and hence limiting the evolution of selfing (Charlesworth & Charlesworth, 1987). However, the degree of inbreeding depression varied among selfing species (Herrera et al., 2001; Michalski & Durka, 2007; Pujol et al., 2009; Rathcke & Real, 1993).

2.3.1 Floral Biology of *Tacca*

Tacca spp. were once believed to attract flies with their dark-coloured, extravagant floral display for cross-pollination (Drenth, 1976). The inflorescence or flowers of *Tacca* spp. possessed the appearance of common sapromyiophilous flowers (Chen et al., 2015; Jersakova et al., 2006). Flies were reported to be attracted to the dull brownish or reddish colour of flowers associated with the scent of decaying substance (Jersakova et al., 2006).

Tacca cristata was reported to emit “a sweetish musky odour” which deceived the insect pollinators to approach the inflorescence (Saw, 1993). This observation was conducted at Forest Research Institute Malaysia (FRIM), Selangor, Malaysia. According to the observation note, the stamen caps and stigmatic lobes were arranged closely, forming only tiny gaps in between. Only flies with size approximately 1 mm able to enter the flower through the gaps. The reproductive structures composed of white, translucent tissues, transmitting light into the interior of the flower. This phenomenon could confuse insects

which had entered the flower when the insects are trying to leave the flower. Thus, the insect movement within the flower unintentionally deposited pollen on the insect or pollinated the flowers while the insect was escaping (Saw, 1993). However, no insect which fulfilled the size requirement visited the flowers during the observation.

Tacca chantrieri was reported to exhibit self-pollination floral characteristics such as anther and stigma nearness in flowers, pollen released before flower opening, and pedicel bending downward resulting in extra chances for self-pollination (Zhang et al., 2005). This observation was also found in several other *Tacca* spp. (Zhang et al., 2007). Similar to *T. cristata*, insect visitors of *T. chantrieri* were reported not effective in pollinating the flowers. Instead, stingless bee (Hymenoptera, Apidae, *Trigona* sp.) which was an opportunistic pollinator was found interacting with stigma and anther as well as collecting pollen. No specialized pollinator was recorded transferring pollen among *T. chantrieri* (Zhang et al., 2005). In addition, the experiments on identifying the function of bracts and bracteoles in pollination failed (Zhang et al., 2005). Removing bracts and reducing the movement of filiform bracteoles showed no significant difference with the controls (Zhang et al., 2005). However, their investigated population had been reduced due to undesirable incidents. Morphologies such as coloured bracts and abundant of bracteoles required high invested resources to be maintained. Owing to the high investment, one would expect useful functions to outweigh the resources allocated.

Fruit and seed set in bagged inflorescence had similar production percentage with open-pollinated inflorescence (Zhang et al., 2005). This species was considered highly selfing as its pollen: ovule (P:O) ratio was low and similar to other self-fertilized species. Furthermore, the estimation of the mating pattern by using molecular polymorphism suggested that a high percentage of seed was produced from self-fertilization (Zhang et al., 2005). These results

led to questions on the development of bracts and bracteoles of *Tacca* spp. as species which are able to self-fertilize tend to adopt a less showy inflorescence (Morgan & Barrett, 1989).

In contrast, another recent study on *T. cristata* at Singapore suggested that cross-pollination exists among *Tacca* spp. (Lim & Raguso, 2017). Contrasting to *T. chantrieri*, various insects (eg. ant, spider, beetle e.t.c.) were recorded visited inflorescence of *T. cristata* with a high portion made up of female ceratopogonid midges (Diptera: Ceratopogonidae). Although the flower did not emit any scent, female midges were observed to be attracted only to the blooming flowers at erecting position. There was pollen deposited on the dorsal parts of midges which visited flowers (Lim & Raguso, 2017). This observation represented the success in pollen transfer from anthers to insects, but the effectiveness of midges in performing cross-pollination between the inflorescences was unresolved. Other than luring pollinators, the conspicuous inflorescence also seems to draw the attention of florivores. A high proportion of inflorescences (40%) had some or all of their flowers consumed (Lim & Raguso, 2017).

2.3.2 Pollinator(s) of *Tacca*

As mentioned in the previous review, the potential pollinator assigned for *T. chantrieri* and *T. cristata* were *Trigona* stingless bee and female ceratopogonid midges, respectively. Although *T. cristata* was visited by a wide range of insect, only midges were observed with pollen deposition (Lim & Raguso, 2017). It is important to understand the habit and behaviour of the insect to clarify its role as the pollinator.

Stingless bees are common pollinator of many flowering plants that occurred in both agricultural and natural environment at tropical and subtropical region (Kwapong et al.,

2010). Within the bee family Apidae, stingless bee belongs to the tribe Meliponini with *Trigona* as the largest genus which is widely distributed in Asia (Heard, 1999). Similar to honey bees, the stingless bee is colonial with a queen, drones and workers (Kwapong et al., 2010). Although this bee has vestigial stings, some species are able to bite enemies to protect and defend their nest and resources (Roubik, 2006; Slaa et al., 2006). Besides *T. chantrieri*, *Trigona* bees were also known to forage pollen from numerous flowering plants with accessible pollen (Nagamitsu et al., 1999). Their swarming behaviours are normally seen in the dry season when the food resources such as nectar, pollen, and other plant substances are abundant (Kwapong et al., 2010). Moreover, stingless bees are active in the morning and seal their nest entrance at dusk (Kwapong et al., 2010). The common foraging distance of stingless bee ranges from 100 to 400 m of the hive's parameter (Heard, 1999).

There were six characteristics of stingless bees which affirm their efficiency as pollinators. First, polylecty of stingless bees contributed to the pollination of numerous plant species as they adapt easily to new introduced flowering plants (Heard, 1999). Yet, second, a stingless bee worker shows floral constancy during a single foraging trip, thus reducing pollen contamination on stigmas of dissimilar species (Ramalho et al., 1994). Third, colonies of stingless bees are known to be perennial in which they constantly develop colonies. And fourth, they store abundant of food resources in their hive. These two behaviours require the stingless bee workers to visit as many flowers as possible, resulting in increased success pollination rate (Heard, 1999; Roubik et al., 1986). Next, stingless bees are believed to perform in-hive pollen transfer similar to the honey bee, so enhancing outcrossing on self-incompatible flowering species (Heard, 1999). Lastly, when workers encounter floral resources, they share the information of location through pheromones, hence, recruiting

ample workers to forage the resources (Heard, 1999; Kwapong et al., 2010). This behaviour thereby increases the pollination success exponentially.

Another recorded pollinator of *Tacca* sp. is female ceratopogonid midges. According to Lim & Raguso (2017), only female midges were observed visited most inflorescences of *T. cristata*. Midges were recorded approached in a zigzag movement, and able to recognise anthetic flowers (erect positioned) instead of post-anthetic flowers (pendent). Their visits were reported to be sporadic, in which some inflorescences attracted a huge amount of midges, while others were not much. Despite, pollen was found only deposited on midges' body rather than other insect visitors (Lim & Raguso, 2017).

Midge is also commonly known as sand fly which is treated as a pest that transmits diseases among livestock and even human through biting (Hill & MacDonald, 2010; Wirth & Hubert, 1989). It is a nuisance as its tiny size allows it to access anywhere and approach the victim without being noticed. In Malaysia, all four subfamilies (Ceratopogoninae, Dasyheleinae, Forcipomyiinae, and Leptoconopinae) and 31 genera were reported occurring in an environment with moisture (Borkent, 2004). However, many species remained unknown due to lack of descriptive information in certain or all life stages of the species. Moreover, adult midges show sexual dimorphism (Burt et al., 1986; McLachlan, 1986). Female ceratopogonid midges typically prey on other insects of equivalent size and feed on the blood of vertebrate including human as a protein source (Borkent, 2004).

Despite of their irritating feeding behaviour, midges were reported as an important pollinator of cocoa tree (Adjaloo & Oduro, 2013; O'Doherty & Zoll, 2012), orchid (Bogarín et al., 2018), aroid (Gibernau et al., 2004) and birthwort (Razzak et al., 1992). Studies found that midges were attracted by volatile esters such as decyl hexanoate, a chemical compound of

pheromone (Jarau et al., 2010; John et al., 2012), and hexyl hexanoate (fruit scent: Sugawara & Muto, 1974). Biting midges also had been proven to be highly attracted to the combination of carbon dioxide, 1-octen-3-ol (octenol) and blue light (Liu et al., 2009). This floral scent lured female midges exclusively but the reason for female midges to be attracted was uncertain. Male midges were rarely observed approaching the flowers or contribute in any pollination. Midges' oviposition on the floral structure was also uncommon in midge attracting flowers (Bogarin et al., 2018). The strategy of flowering plants which attracted midges for pollination was suggested as kleptomyiophilous (Bogarin et al., 2018). This pollination syndrome deceives kleptoparasitic insects which steal and feed on the prey of other predators. Kleptomyiophilous flowers produce mimicry scent of insect venom or freshly killed insect fooling flies which are in search of food (Heiduk et al., 2015; Oelschlagel et al., 2014). Few species of ceratopogonid midges were known to be kleptoparasites (Marshall et al., 2015).

2.4 Phytochemical constituents of *Tacca*

Tubers of *Tacca* spp. were recorded to be consumed as food by indigenous people of Polynesia especially Tahiti and Java when staple foods are deficient (Kay, 1987; Wardah, 2017). Simple poison removal method was needed as the tubers are poisonous. The process of eliminating poison included repeatedly washing and rinsing the tubers with water before consuming the tubers (Borokini & Ayodele, 2012; Bosha et al., 2015). In addition, Jiang and colleagues (2014) reported that amylose content of *Tacca* starch is higher than maize which further confirms the potential of *Tacca* tubers on replacing staple food crops such as potatoes.

Starch from *T. leontopetaloides* also had been recorded to be processed into flour as raw material for producing various food products (Wardah, 2017).

Other than eaten as food, the nutritional value of *Tacca* spp. is also relatively high despite some toxic present in the marc of *Tacca* tubers (Bosha et al., 2015). Vitamin analysis revealed the occurrence of Vitamin A, B, C and E which act as antioxidant in the human body to reduce the risk of chronic diseases. Essential elements such as potassium and sodium were found in *T. leontopetaloides*'s rhizome (Bosha et al., 2015). These elements act as important electrolytes in balancing the cellular homeostasis including osmotic pressure, oxidation-reduction reactions and cofactor catalysis of enzymes (Pohl et al., 2013). According to Bosha and colleagues (2015), beneficial components such as iron, zinc, and magnesium also been tested to present in trace amount in *Tacca* spp.'s marc.

Tacca spp. were also being reported to have pharmacological potentials as most parts of *Tacca* plants were used as traditional medicines (Borokini & Ayodele, 2012; Bosha et al., 2015; Jiang et al., 2014; Williams, 2012). Among inhabitant, the roots and flowers of *Tacca* spp. were sometimes used to treat snakebite (Bosha et al., 2015). From the extraction of phytochemicals from *Tacca* spp., various chemicals including flavonoid, alkaloid and tannin were discovered (Borokini & Ayodele, 2012; Bosha et al., 2015). About 35 different chemical structures of taccalonolides were extracted in research done by Jiang et al. (2014). These natural steroidal glycosides perform microtubule-stabilizing activity which is also observed in the most efficient anticancer drugs, Paclitaxel (Jiang et al., 2014). Besides that, some tannins and saponins which are also one of the chemical constituents are known to have the ability to suppress cancer cell's growth (Guclu-Ustundag & Mazza, 2007; Okuda & Ito, 2011).

However, unfavourable components also exist in several plant parts of *Tacca* spp. For instances, heavy metals were extracted from the marc of *T. involucrata* Schumach. & Thonn. (synonym of *T. leontopetaloides*) by Bosha and colleagues (2015) but in low quantity which is not beyond the recommended daily consumption limit. Accumulation of heavy metals such as lead, mercury and arsenic which were also reported in Bosha and colleagues' research (2015) can incur damage to the human body (National Organization for Rare Disorders, 2006). Moreover, the peels of *Tacca* spp.' tubers contain high anti-nutritional factors (Ubwa et al., 2011). Unprocessed tubers are not consumable by human and livestock as the peels are poisonous. Result of experiments done by Ubwa and colleagues stated that *T. leontopetaloides* peels contained phytate, oxalate, cyanogenic glycoside and haemoglutinin (Ubwa et al., 2011). These compounds were described to cause varying health concerns to a certain degree.

Although flowers of *T. cristata* were reported to emit musky odour (Saw, 1993), no floral scent collection was conducted to analyse the odour chemical compound. Other observations did not detect any floral scent (Lim & Raguso, 2017).

CHAPTER 3

PHYLOGENETIC AND MORPHOLOGICAL ANALYSIS OF *Tacca*

3.1 Introduction

In order to understand the evolution of inflorescence traits, phylogenetic reconstructions of genus *Tacca* were performed in this study. The previous phylogenetic study on *Tacca* spp. did not involve any species/accessions from Borneo. In this study, all known *Tacca* spp. from Sarawak were included to assess the ancestral state and evolution direction of genus *Tacca*'s morphologies especially the conspicuous inflorescence. The gene regions used were nuclear internal transcribed spacer (ITS) and protein-coding chloroplast maturase K (matK). Morphology of *Tacca* spp., either vegetative or reproductive, varies greatly among species. Not only their plant habit can be divided into annual and perennial, species such as *T. palmata* have palmate leaf shape whereas more than half of the other *Tacca* spp. have long lanceolate leaf blade. The floral characteristics (e.g. bract shape and size, bracteole number) are also distinctive for differentiating *Tacca* spp. The previous report on morphological characters mapping concluded the *Tacca* spp. evolved from less showy inflorescence structures to conspicuous bracts and bracteoles (Zhang et al., 2011). However, the study did not include *Tacca* spp. in Borneo which develop small linear bracts such as *T. reducta* and *T. bibracteata*.

3.2 Materials and methods

3.2.1 Phylogenetic analysis

3.2.1.1 Taxon sampling

In this study, 16 species out of c. 20 known species were included from various localities. The representative species were: *T. amplipecta*, *T. bibracteata*, *T. borneensis*, *T. chantrieri*, *T. cristata*, *T. integrifolia*, *T. laevis*, *T. leontopetaloides*, *T. maculata*, *T. palmata*, *T. palmatifida*, *T. parkeri*, *T. plantaginea*, *T. reducta*, *T. subflabellata* and *T. sumatrana*, as well as several unplaced species. Twenty-nine of the samples were collected from Malaysia, especially Sarawak. The study also included taxa from Cairns Botanic Gardens (nine taxa), India (one taxon), Indonesia (three taxa), Thailand (one taxon) and Vietnam (one taxon). ITS and matK sequences of nine *Tacca* spp. (*T. amplipecta*, *T. chantrieri*, *T. cristata*, *T. integrifolia*, *T. leontopetaloides*, *T. palmata*, *T. palmatifida*, *T. plantaginea* and *T. subflabellata*) were downloaded from NCBI. For *T. parkeri*, only ITS sequence was available from (NCBI). Four *Tacca* spp., namely *T. ankaranensis*, *T. celebica*, *T. ebeltajae* and *T. khamhhaensis*, were not included in this study. Sequences of *Trichopus sempervirens* from Trichopodaceae were downloaded as the outgroup species because of their close affinities within Dioscoreales (Caddick et al., 2002; Couto, 2017; Wilkin et al., 2005; Zhang et al., 2011).

Eighty-eight new sequences were generated from 44 accessions of 12 *Tacca* spp. together with 21 sequences downloaded from genbank. Details of all sequences included in the analyses were listed in Table 3.1.

Table 3.1: List of sampled taxa, their voucher, locality, and accession number in phylogenetic analyses.

Taxon	Voucher	Genbank accession number	
		ITS	matK
<i>Tacca bibracteata</i>	MALAYSIA. Sarawak: Sri Aman, Lubok Antu, 30 Mar 2018, Wong Sin Yeng & Peter C. Boyce TA96 (SAR).	MK144508	MK153225
<i>Tacca borneensis</i>	MALAYSIA: Sarawak, Sarikei, Bayong, Ulu Sarikei, 3 Jun 2017, Wong Sin Yeng & Peter C. Boyce TA67 (SAR).	MK144485	MK153202
<i>Tacca borneensis</i>	MALAYSIA: Sarawak, Kapit, Seabai, 30 Nov 2017, Chua Kek Shen TA77 (SAR).	MK144501	MK153218
<i>Tacca borneensis</i>	MALAYSIA: Sarawak, Kuching, Bau, Bungo Range, 7 Dec 2017, Chua Kek Shen TA79 (SAR).	MK144502	MK153219
<i>Tacca borneensis</i>	MALAYSIA: Sarawak, Kuching, Matang, Kubah National Park, Sep 2017, Chua Kek Shen TA84 (SAR).	MK144491	MK153209
<i>Tacca borneensis</i>	MALAYSIA: Sarawak, Kuching, Bau, Rajah Brooke Heritage, Sep 2017, Chua Kek Shen TA86 (SAR).	MK144489	MK153207
<i>Tacca borneensis</i>	MALAYSIA: Sarawak, Kuching, Padawan, Borneo Highland, Sep 2017, Chua Kek Shen TA89 (SAR).	MK144490	MK153208
<i>Tacca chantrieri</i>	Cairns Botanic Gardens, Steve Jackson TA63.	MK144482	MK153199
<i>Tacca chantrieri</i>	MALAYSIA: Selangor, Kuala Lumpur, Bukit Nanas Forest Reserve, Sep 2017, Chua Kek Shen TA91 (SAR).	MK144495	MK153213
<i>Tacca chantrieri</i>	VIETNAM: Kien Giang, Leong, Rach Gia, 1 Jun 2018, David Scherberich TA106.	MK144513	MK153230
<i>Tacca cristata</i>	MALAYSIA. Selangor: Gombak, FRIM, 17 Dec 2016, Wong Sin Yeng & Peter C. Boyce TA55 (SAR).	MK144505	MK153222
<i>Tacca cristata</i>	MALAYSIA: Selangor, Kuala Lumpur, Bukit Nanas Forest Reserve, Jan 2018, Chua Kek Shen TA109 (SAR).	MK144506	MK153223
<i>Tacca integrifolia</i>	INDIA: Arunachal Pradesh, 4 Nov 2016, Pascal Bruggeman TA48 (SAR).	MK144516	MK153233

Table 3.1 continued

<i>Tacca integrifolia</i>	Cairns Botanic Gardens, Steve Jackson TA59.	MK144478	MK153195
<i>Tacca integrifolia</i>	Cairns Botanic Gardens, Steve Jackson TA62.	MK144481	MK153198
<i>Tacca laevis</i>	MALAYSIA: Sarawak, Kuching, Matang, Sungai Adis, 1 Jul 2017, Chua Kek Shen TA73 (SAR).	MK144492	MK153210
<i>Tacca laevis</i>	MALAYSIA: Sarawak, Kuching, Matang, Kubah National Park, Sep 2017, Chua Kek Shen TA88 (SAR).	MK144493	MK153211
<i>Tacca laevis</i>	MALAYSIA: Sarawak, Kuching, Bau, Kampung Segong, Nov 2017, Chua Kek Shen TA92 (SAR).	MK144518	MK153235
<i>Tacca laevis</i>	MALAYSIA: Sabah, Sandakan, Sepilok, 6 Feb 2018, Wong Sin Yeng & Peter C. Boyce TA93 (SAR).	MK144503	MK153220
<i>Tacca leontopetaloides</i>	Cairns Botanic Gardens, Steve Jackson TA60.	MK144479	MK153196
<i>Tacca leontopetaloides</i>	MALAYSIA: Sarawak, Kuching, Lundu, Sampadi, Sep 2017, Chua Kek Shen TA81 (SAR).	MK144494	MK153212
<i>Tacca maculata</i>	Cairns Botanic Gardens, Steve Jackson TA61.	MK144480	MK153197
<i>Tacca palmata</i>	MALAYSIA. Sabah: Kudat, Kota Murudu, 11 Feb 2017, Wong Sin Yeng & Peter C. Boyce TA56 (SAR).	MK144475	MK153192
<i>Tacca palmata</i>	Cairns Botanic Gardens, Steve Jackson TA64.	MK144483	MK153200
<i>Tacca palmata</i>	INDONESIA: Kalimantan Barat, Bengkayang, Pajintan, 2 Sep 2017, Wong Sin Yeng & Peter C. Boyce TA75 (SAR).	MK144486	MK153204
<i>Tacca palmata</i>	MALAYSIA: Sarawak, Kuching, Lundu, Sampadi, Sep 2017, Chua Kek Shen TA80 (SAR).	MK144496	MK153214
<i>Tacca palmata</i>	MALAYSIA: Sarawak, Kuching, Serian, Taman Rekreasi Ranchan, Sep 2017, Chua Kek Shen TA83 (SAR).	MK144499	MK153217
<i>Tacca palmata</i>	MALAYSIA: Sarawak, Kuching, Bau, Rajah Brooke Heritage, Sep 2017, Chua Kek Shen TA87 (SAR).	MK144488	MK153206
<i>Tacca palmata</i>	MALAYSIA: Sarawak, Kuching, Matang, Sungai Adis, Sep 2017, Chua Kek Shen TA90 (SAR).	MK144497	MK153215
<i>Tacca plantaginea</i>	Cairns Botanic Gardens, Steve Jackson TA65.	MK144484	MK153201

Table 3.1 continued

<i>Tacca reducta</i>	MALAYSIA: Sarawak, Kuching, Serian, Taman Rekreasi Ranchan, Sep 2017, Chua Kek Shen TA82 (SAR).	MK144498	MK153216
<i>Tacca reducta</i>	MALAYSIA: Sarawak, Kuching, Bau, Rajah Brooke Heritage, Sep 2017, Chua Kek Shen TA85 (SAR).	MK144487	MK153205
<i>Tacca sumatrana</i>	INDONESIA: North Sumatera, Toba Samosir, Lok Lok Pan, 31 Jan 2018, Pascal Bruggeman TA95.	MK144507	MK153224
<i>Tacca</i> sp.	MALAYSIA: Kelantan, Tanah Merah, Hulu Kusial, Guai Ipoh, 16 Mar 2016, Wong Sin Yeng & Peter C. Boyce TA46 (SAR).	MK144515	MK153232
<i>Tacca</i> sp.	Cairns Botanic Gardens, Steve Jackson TA57.	MK144476	MK153193
<i>Tacca</i> sp.	Cairns Botanic Gardens, Steve Jackson TA58.	MK144477	MK153194
<i>Tacca</i> sp.	MALAYSIA: Sarawak, Sarikei, Bayong, Ulu Sarikei, 3 Jun 2017, Wong Sin Yeng & Peter C. Boyce TA68 (SAR).	MK144500	MK153203
<i>Tacca</i> sp.	MALAYSIA: Perak, Ipoh, Bercham, 20 Jun 2017, Lim Teow Yeong TA70 (SAR).	MK144517	MK153234
<i>Tacca</i> sp.	MALAYSIA: Sabah, Sandakan, Sepilok, 6 Feb 2018, Wong Sin Yeng & Peter C. Boyce TA94 (SAR).	MK144504	MK153221
<i>Tacca</i> sp.	INDONESIA: Jawa Barat, Sukabumi, Pasawahan, 24 May 2018, Albert Doang TA102.	MK144509	MK153226
<i>Tacca</i> sp.	MALAYSIA: Sabah, Kota Kinabalu, Inanam, 27 May 2018, Wong Sin Yeng & Peter C. Boyce TA103 (SAR).	MK144510	MK153227
<i>Tacca</i> sp.	MALAYSIA: Sabah, Tuaran, Tamparuli, 28 May 2018, Wong Sin Yeng & Peter C. Boyce TA104 (SAR).	MK144511	MK153228
<i>Tacca</i> sp.	THAILAND: Satun, Thaleban, 1 Jun 2018, David Scherberich TA105.	MK144512	MK153229
<i>Tacca</i> sp.	MALAYSIA: Sabah, Tawau, Lahad Datu, 7 Jul 2018, Wong Sin Yeng & Peter C. Boyce TA108 (SAR).	MK144514	MK153231

Table 3.1 continued

<i>Tacca ampliplacenta</i>	ITS: Zhang et al. (2011) matK: Li et al. (2011)	JN850566.1	JF956618.1
<i>Tacca chantrieri</i>	ITS: Li et al. (2011) matK: Li et al. (2011)	JF978861.1	JF956623.1
<i>Tacca integrifolia</i>	ITS: Zhang et al. (2011) matK: Li et al. (2011)	JN850569.1	JF956637.1
<i>Tacca cristata</i>	ITS: Zhang et al. (2011) matK: Wilkin et al. (2005)	JN850568.1	AY973838.1
<i>Tacca leontopetaloides</i>	ITS: Zhang et al. (2011) matK: Wilkin et al. (2005)	JN850570.1	AY973839.1
<i>Tacca palmata</i>	ITS: Zhang et al. (2011) matK: Wilkin et al. (2005)	JN850571.1	AY973840.1
<i>Tacca palmatifida</i>	ITS: Zhang et al. (2011) matK: Wilkin et al. (2005)	JN850572.1	AY973841.1
<i>Tacca parkeri</i>	ITS: Zhang et al. (2011) matK: -	JN850573.1	-
<i>Tacca plantaginea</i>	ITS: Zhang et al. (2011) matK: Wilkin et al. (2005)	JN850574.1	AY973842.1
<i>Tacca subflabellata</i>	ITS: Zhang et al. (2011) matK: Li et al. (2011)	JN850575.1	JF956654.1
<i>Trichopus sempervirens</i>	ITS: Zhang et al. (2011) matK: Hertweck et al. (2015)	JN850576.1	KP083035.1

3.2.1.2 DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from c. 0.1 g fresh, or silica-gel dried leaves for all accessions, using DNeasy Plant MiniKit (Qiagen, Hilden, Germany) according to the manufacturer's manual. The samples were frozen under temperature – 80 °C to be crushed into powder. The extracted DNA was stored at – 20 °C for amplification.

Double-stranded DNA was directly amplified by polymerase chain reaction (PCR) amplification. Three pairs of primers were used for amplifying two gene regions, ITS and matK. For ITS, forward primer 18F (5'-GTC CAC TGA ACC TTA TCA TTT AGA GG-3') (Kass & Wink, 1997, modified by Beyra-Matos & Lavin, 1999) and reverse primer 26R (5'-GCC GTT ACT AAG GGA ATC CTT GTT AG-3') (Kass & Wink, 1997) were used. For

matK gene region, two pairs of primers were used which were the 19F (5'-CGT TCT GAC CAT ATT GCA CTA TG-3') with 1326R (5'-TCT AGC ACA CGA AAG TCG AAG T-3') primers of Molvray et al. (2000) and Cuenoud et al. (2002), and 390F (5'-CGA TCT ATT CAT TCA ATA TTT C-3') with 2R (5'-AAC TAG TCG GAT GGA GTA G-3') primers of Cuenoud et al. (2002) and Johnson & Soltis (1994).

The total reaction volumes were 20 μ L. For amplifying ITS region, the PCR mix contained 4.0 μ L 10X buffer, 2.0 μ L DNA, 0.4 μ L primer (10 mM), 0.2 μ L dNTP (10 mM), 1.6 μ L $MgCl_2$ (25 mM), 0.2 μ L Taq polymerase (PROMEGA), 1 μ L dimethyl sulfoxide (DMSO), and double distilled water was added to 20 μ L. For amplifying matK region, the PCR mix contained 4.0 μ L 10X buffer, 2.0 μ L DNA, 0.4 μ L primer (10 mM), 0.2 μ L dNTP (10 mM), 1.6 μ L $MgCl_2$ (25 mM), 0.2 μ L Taq polymerase (PROMEGA), and double distilled water was added to 20 μ L. PCR was performed in a T100 Thermal Cycler (BIO-RAD). The PCR process began DNA denaturation with 2 mins at 95 °C at first, followed by 30 cycles of 1 min at 95 °C for template denaturation, 1 min at 50 °C or 52 °C for primer annealing, 2 mins at 72 °C for primer extension, lastly followed by a final extension of 10 min at 72 °C. Negative controls, which were not inserted with DNA, were included in each PCR process to test for the presence of contamination.

3.0 μ L of PCR products were co-loaded with stain into 1.0 % (w/v) agarose gels with SYBR Safe DNA gel stain in 1X TAE buffer at 80V for 10 – 15 mins along with a 100-1500bp ladder (First BASE Laboratories Sdn. Bhd.). After the electrophoresis, the DNA bands were visualized on a non-UV side illuminator fluorescent PrepOne™ Sapphire (Embi Tec). The remaining PCR products were stored at 4°C until use. If DNA band was present, PCR was repeated another five times for each sample to obtain an accumulated PCR product with a volume approximately 100 μ L.

Final PCR products were purified using GeneJET purification kit prior to sequencing. Then, purified PCR reactions were sent to Apical Scientific Sdn. Bhd. at Selangor, Malaysia for sequencing.

3.2.1.3 Sequence editing and alignment

The software BioEdit version 6.0.7 (Hall, 2004) was used to edit and assemble the complementary strands of the sequencing results. This software allowed comparison of the DNA chromatogram for both forward and reverse sequences, and hence eased the combination of the sequences. All the sequences were saved in FASTA format. The sequences were uploaded to web Basic Local Alignment Search Tool (BLAST) to find the resemblance nucleotide sequences within network service databases (NCBI, 2018). The multiple sequence alignments were generated in ClustalX version 2.0 (Larkin, 2007) for ITS region, matK region and combined dataset of both regions.

3.2.1.4 Phylogenetic analyses

Phylogenetic analyses were conducted using maximum parsimony (MP), maximum likelihood (ML) and bayesian inference (BI) to estimate tree topologies based on three datasets (ITS, matK and combined sequences). MP was implemented in PAUP version 4.0a163 (Swofford, 2002). For ML analysis, the sequence files were uploaded to online RAxML Blackbox version 0.6.0 (Kozlov et al., 2008). BI phylogenetic trees were inferred in MrBayes version 3.2.6 (Huelsenbeck & Ronquist, 2001) for all datasets. For ITS analyses, the phylogenetic tree presented was based on BI analysis because the resulting topologies for each data sets were consistent among analyses except for ML analysis in ITS. Whereas,

ML trees were used to present the topologies of analyses of both matK and combined dataset. Other phylogenetic trees were placed in Appendix A.

In MP analysis, characters were treated as unordered and equally weighted. Heuristic searches were computed with 1000 jackknife replicates and automatically generated starting seed as well as 50 % nominal deletion percentage. The starting tree was obtained via stepwise addition with 10 random replicates. Tree bisection reconnection (TBR) was set with reconnection limit equal to 8, and 1 tree was held at each step. “MulTrees” option was in effect. Branches were collapsed creating polytomies if the supporting value was less than 50. The consistency index (CI) and retention index (RI) for each dataset were also recorded from PAUP.

jModelTest version 2.1.9 (Darriba et al., 2012) was used to choose the best nucleotide substitution model and parameter settings for each aligned datasets in ML and BI analyses by using the Akaike information criterion (AIC). For computing ML analysis, the TPM1uf(K81uf)+G model and the TIM3+G model were selected for matK region and ITS region, respectively. For BI analysis, ITS and matK region were analysed with the GTR + G model.

For the ML analyses, optimal gene trees were searched by optimizing topology, branch lengths and model. 100 bootstrapping replicates of random sequences were estimated. Analysis began with 10 parsimony trees and 10 randomly generated trees. For the combined dataset, the analysis was run with a partitioned model since both of the regions had different best nucleotide substitution models recommended by jModelTest.

Bayesian inference was conducted in MrBayes based on the model suggested by jModelTest for each gene regions. Four independent Markov Chain Monte Carlo (MCMC) chains were

set with one cold chain and three heated chains (temperature = 0.1). The MCMC chains were run simultaneously and sampled every 500 generations for a total of 1 000 000 generations. First 10% of the 2001 sampled trees were discarded as burn-in. Other parameter settings were the default.

Strict consensus trees from MP analysis were observed and converted into an image file by using TreeView version 1.6.6 (Page, 1996). FigTree version 1.4.3 (Rambaut, 2016) was used to open and save trees produced in ML and BI analyses. All phylogeny trees editing was conducted in PhoXo image editor version 8.3.0 (Fu, 2014). Bootstrap (BS) or posterior probability (PP) values of less than 50% or 0.50 were not presented on the resulting phylogeny trees. Branches with BS or PP values of 85 – 100% or 0.85 – 1.00 are regarded as strongly supported.

3.2.2 Morphological analysis

3.2.2.1 Taxon sampling

The combined dataset of both ITS and matK sequences was analysed using MP method by PAUP to generate the base tree for morphological characters mapping. Each species was represented by one combined sequence of the two gene regions. The accession included were *T. bibracteata* (TA96), *T. borneensis* (TA84), *T. chantrieri* (TA106), *T. cristata* (TA55), *T. integrifolia* (TA48), *T. laevis* (TA92), *T. leontopetaloides* (TA81), *T. palmata* (TA83), *T. plantaginea* (TA65), *T. reducta* (TA82), and *T. sumatrana* (TA95). *Tacca amplipecta*, *T. palmatifida* and *T. subflabellata* were also added with their sequences downloaded from NCBI genbank. The total amount of *Tacca* spp. analysed was 14 species. *Trichopus sempervirens* was added to root the tree.

3.2.2.2 Mapping of morphological characters onto the phylogenetic tree

The majority of morphologies mapped onto the phylogenetic tree was floral characteristics. This characters included the length of peduncle, size of inner and outer involucral bracts, number of inner bracts, colour of bracts, number of bracteoles, length of bracteoles, number of flowers, and number of ovules. Moreover, the leaf shape was added as the vegetative character. Information on the length of bracts and bracteoles were measured using a measuring tape. The species which were measured are *T. bibracteata*, *T. borneensis*, *T. cristata*, *T. integrifolia*, *T. laevis*, *T. leontopetaloides*, *T. reducta*, and *T. sumatrana* (Table 3.2). Data for the rest of the species were obtained from the descriptions of published reports. Average length and width of bracts were calculated by summation of minimum and maximum recorded values and divided by 2. Measurements on bract sizes were estimated by multiplication of average length and width of the bracts. The average length of bracteoles was also deduced using the calculation method for length and width of bracts. The mean number of bracteoles were obtained for species in Borneo, whereas data on bracteoles number for other species was the half of the summation of minimum and maximum values from previous publications.

Table 3.2: The number of inflorescence measured for data of quantitative morphological characters for each species.

Species	Number of inflorescence replicates
<i>T. bibracteata</i>	3
<i>T. borneensis</i>	4
<i>T. cristata</i>	2
<i>T. integrifolia</i>	1
<i>T. laevis</i>	4
<i>T. leontopetaloides</i>	6
<i>T. reducta</i>	5
<i>T. sumatrana</i>	1

All of the morphological characters were mapped with Mesquite version 3.51 (Maddison & Maddison, 2018), which allows us to view changes in these characters on a parsimonious tree. The inflorescence traits for each species were focused to analyse the evolution and ancestral states of *Tacca* floral characteristics. The types of characters were categorised into numeric matrix and “0” indicates no data for the species on the morphology (Table 3.3).

Table 3.3: The included morphology characters and the respective description of character matrices used to map the phylogenetic tree.

Morphology characters	Character matrices			
	1	2	3	4
Leaf shape	Palmate	Entire	-	-
Length of peduncle	≤ 30 cm	31 – 60cm	> 60 cm	-
Size of inner bracts	≤ 20 cm ²	21 – 40 cm ²	41 – 60 cm ²	> 60 cm ²
Size of outer bracts	≤ 20 cm ²	21 – 40 cm ²	41 – 60 cm ²	> 60 cm ²
Number of inner bracts	2	> 2	-	-
Colour of bracts	Green	Other colour	-	-
Number of bracteoles	0	1 – 10	11 – 20	> 20
Length of bracteoles	≤ 12	12 – 22	> 22	-
Number of flowers	≤ 10	11 – 20	> 20	-
Number of ovules	≤ 40	41 – 100	101 – 200	> 200

3.3 Results and discussion

3.3.1 Phylogenetic reconstruction

The unaligned sequences of the nuclear ITS and cytoplasmic matK region of the ingroup taxa ranged from 719 to 760 base pairs (bp) and 1681 to 1732 bp, respectively. A total of 830 characters were aligned for ITS, in which 441 (53.13%) characters were constant, 121 (14.58%) variable characters were parsimony uninformative, and 268 (32.29%) parsimony-informative characters. Tree length for this gene region was 674 steps with CI = 0.769 and RI = 0.924. For matK, out of 1766 total aligned characters, there were 1376 (77.92%) constant characters, 253 (14.33%) variable characters, and 137 (7.76%) parsimony-informative characters. Tree of matK region had a length of 446 steps with CI = 0.924 and RI = 0.955. The final alignment for the combined sequences for the two regions comprised 2596 characters which included 1817 (69.99%) constant sites, 377 (14.52%) variable sites, and 402 (15.49%) parsimony informative sites. Combined dataset had tree length = 1040 with CI = 0.817 and RI = 0.923. Table 3.4 summarized the information of tree support for all datasets.

Table 3.4: Statistics of tree support according to the analysis of data sets in PAUP.

DNA region	No. of taxa	No. of characters	No. of variable sites	No. of informative sites	Tree length	CI	RI
ITS	54	830	121	268	674	0.769	0.924
matK	53	1766	253	137	446	0.924	0.955
Combine	53	2596	377	402	1040	0.817	0.923

CI, consistency index; RI, retention index.

The tree topologies of ITS and matK regions yielded a slightly incongruent result for all analyses (Figure 3.1 & 3.2). Trees generated from combined dataset agreed with the topologies of ITS (Figure 3.3). Figure 3.4 shown the differences in tree topologies of MP analysis among different data sets. The combined dataset of two gene regions (ITS and matK) yielded a result congruent with the phylogenetic analysis of the combination of five gene regions (ITS, atpA, rbcL, trnL-F, and trnH-psbA; Zhang et al., 2011).

For all phylogenetic trees except ML for ITS, *T. leontopetaloides*, which was a geographically widespread species, and *T. maculata* formed the basal clade to all other *Tacca* species. But, the clade with *T. integrifolia* and *T. amplioplacenta* was sister to the other species in ITS ML tree instead of the clade with *T. leontopetaloides* and *T. maculata*. However, the clade for ITS ML tree had insignificant supporting value (lower than 50), leading to the doubt of its topological acceptance. *Tacca palmata*, regardless of their origin, were forming a clade together with *T. palmatifida* in all analyses with strong supporting values (BS > 85, PP = 1.00) except ITS ML analysis. The only New World species, *T. parkeri*, and *T. plantaginea* from Old World region were found to be in a clade in ITS phylogenetic trees with high supporting values (BS > 91, PP = 0.98). *Tacca bibracteata*, which is endemic to Borneo, was either remained unresolved or found in a clade with *T. palmata*, *T. palmatifida*, and *T. plantaginea*. The long branch length of subclade with *T. plantaginea* in matK was observed (Figure 3.2). The doubt of the sequence being a pseudogene can be excluded as one of the sequences was from a published source, and the generated sequence (TA65) was cross-checked on BLAST (NCBI, 2018) with other matK sequences of *Tacca* in which the similarity percentage was more than 90%. Moreover, the number of bp for TA65 (1727 bp) did not differ much from other sequences (1681 – 1732 bp). Taxonomic position of *T. plantaginea* was not certain as it was previously recognised

under genus *Schizocapsa* with dehiscence fruits which this character did not appear in other *Tacca* spp. (Hassan & Uddin, 2007).

Tacca integrifolia formed a clade with *T. amplipecta* in ITS analysis, but from the matK analysis, *T. integrifolia* joined a clade with *T. subflabellata*. Both of these clades had high supporting values (ITS: BS > 93, PP = 1.00; matK: BS > 86, PP = 1.00). In ITS analysis, all *T. chantrieri* sequences and *T. subflabellata* formed a clade. Yet, *T. chantrieri* was placed together with *T. amplipecta* in an unresolved clade in matK analysis. Despite the lack of literature on *T. amplipecta* and *T. subflabellata*, there were similarities in their species descriptions. Both *T. amplipecta* and *T. subflabellata* were originated from Yunnan province, southwestern China (Ling & Ting, 1982; Zhang & Li, 2008a). Morphologies of these two species were recorded to be closely resembling *T. chantrieri*. Moreover, *T. amplipecta* was suggested to be the hybrid of outcrossing between *T. chantrieri* and *T. integrifolia* (Zhang & Li, 2008a). Hence, this might be the cause of incongruence in the position of *T. amplipecta* and *T. subflabellata* between analysis of ITS and matK.

A *Tacca* sp. (TA70) belonged to the clade with *T. chantrieri* in all analyses. The accession sample TA70 was originated from Perak, Malaysia (North part of Peninsular Malaysia). Morphologies of TA70 concurred to the description of *T. chantrieri* (Drenth, 1976). However, the huge morphological variations owing to synonymization of at least five taxa under *T. chantrieri* made the description unreliable. In green house (surrounded by natural lowland forest), only TA70 was frequently observed with infructescences which implied that either there is pollinator attracted merely to the inflorescence of TA70 or it performs autogamous self-pollination. As reported by Zhang et al. (2005), *T. chantrieri* depended highly on autogamous self-pollination to achieve reproductive success. This was probably the reason for the position of TA70 within a clade with *T. chantrieri*.

Next, all accessions of *T. borneensis* were placed in a clade of high support values (BS > 84, PP > 0.99). These accessions formed a clade regardless of their original localities within Sarawak. Three unknown samples of *Tacca* from Sabah with accession number TA94, TA103 and TA108 were placed into the clade with *T. borneensis*. The accessions were either unresolved (matK) or formed a subclade (ITS & combined) with moderate to strong support values (BS = 75 – 94, PP = 1.00).

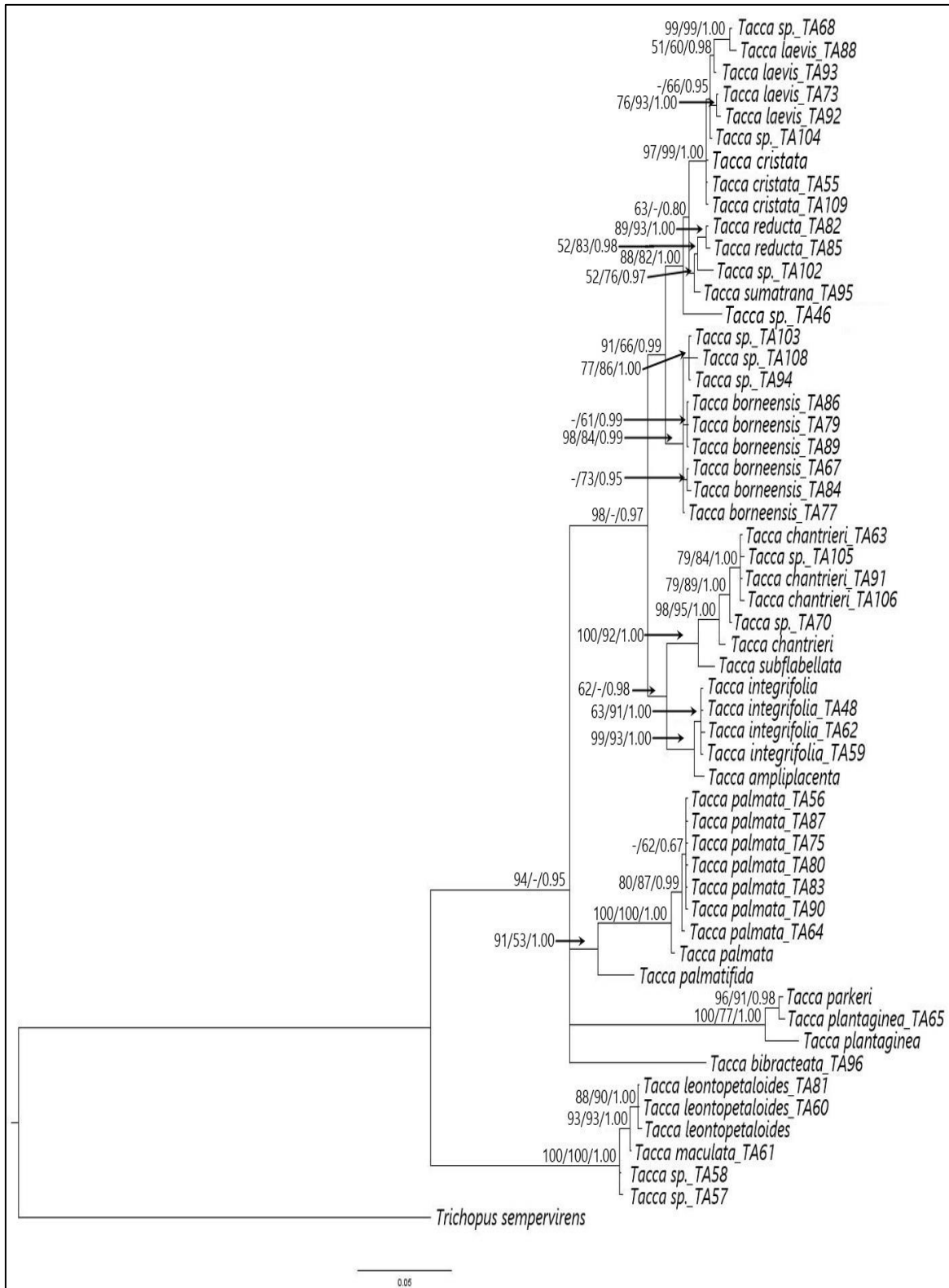


Figure 3.1: Phylogenetic relationships of *Tacca* spp. according to BI analysis of ITS region with supporting values above the branch (numbers from left to right indicate MP BS, ML BS, and BI PP; “-” indicate BS values less than 50).

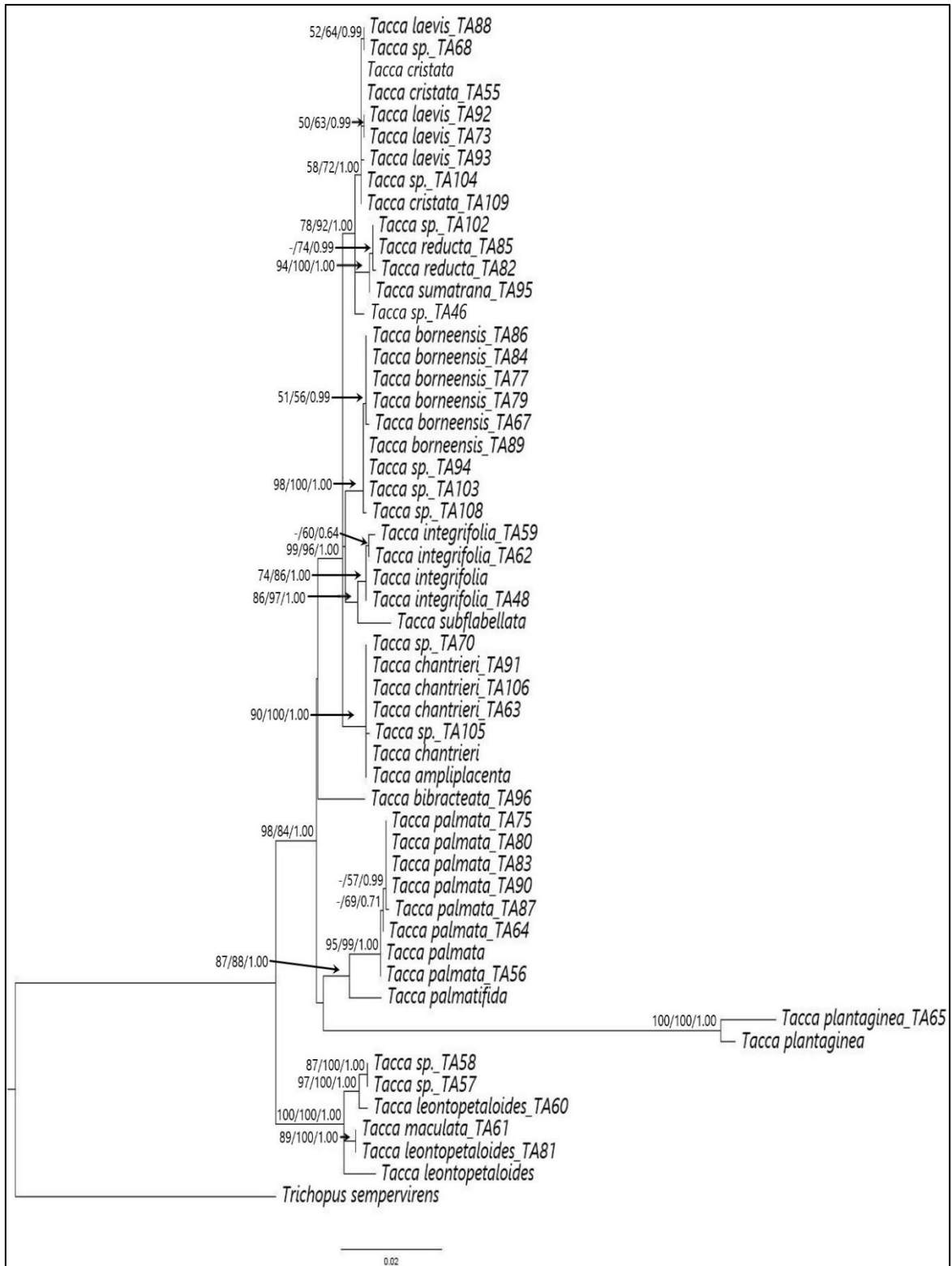


Figure 3.2: Phylogenetic relationships of *Tacca* spp. according to ML analysis of matK region with supporting values above the branch (numbers from left to right indicate MP BS, ML BS, and BI PP; “-” indicate BS values less than 50).

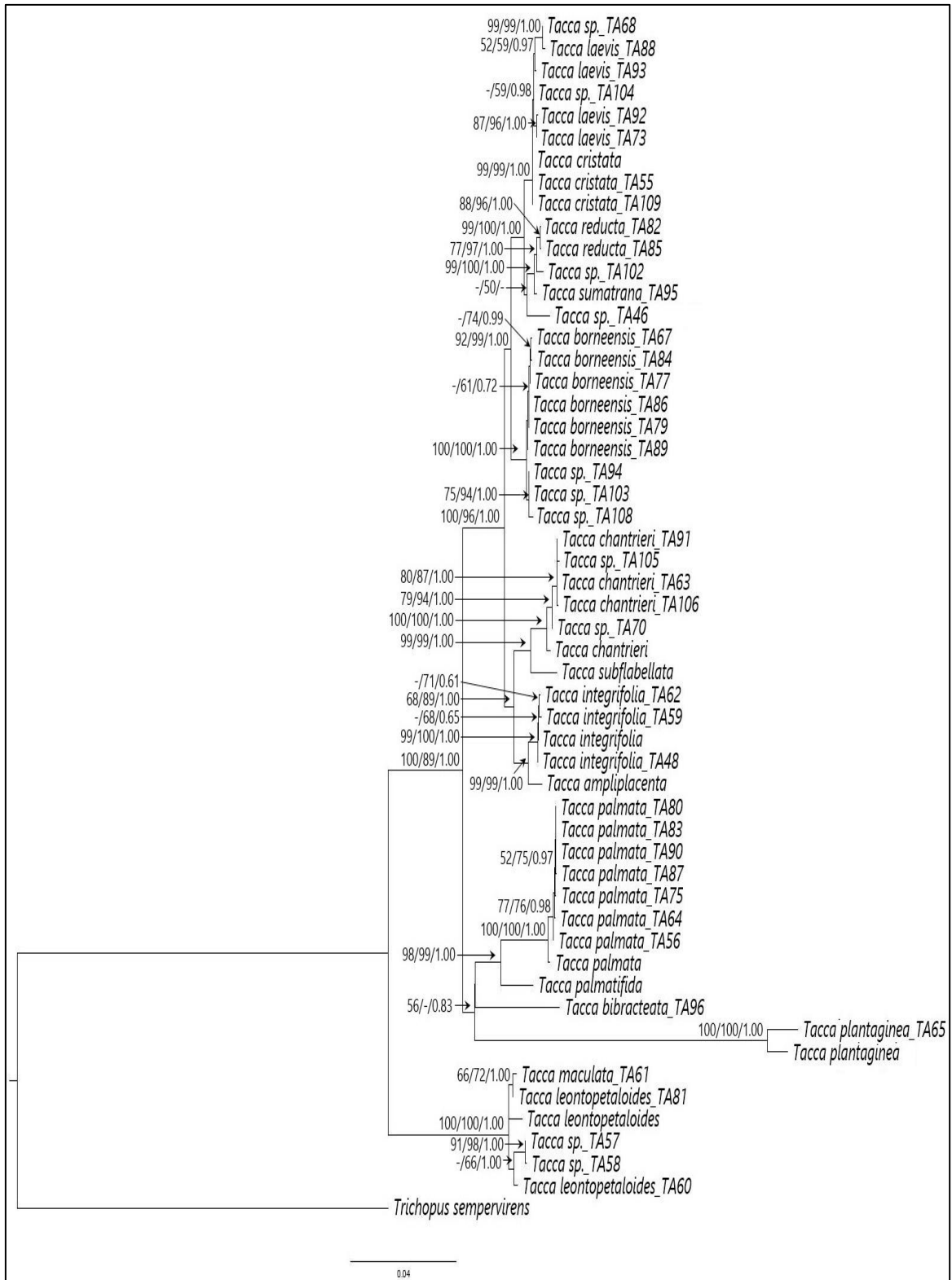


Figure 3.3: Phylogenetic relationships of *Tacca* spp. according to ML analysis of combined data sets with supporting values above the branch (numbers from left to right indicate MP BS, ML BS, and BI PP; “-” indicate BS values less than 50).

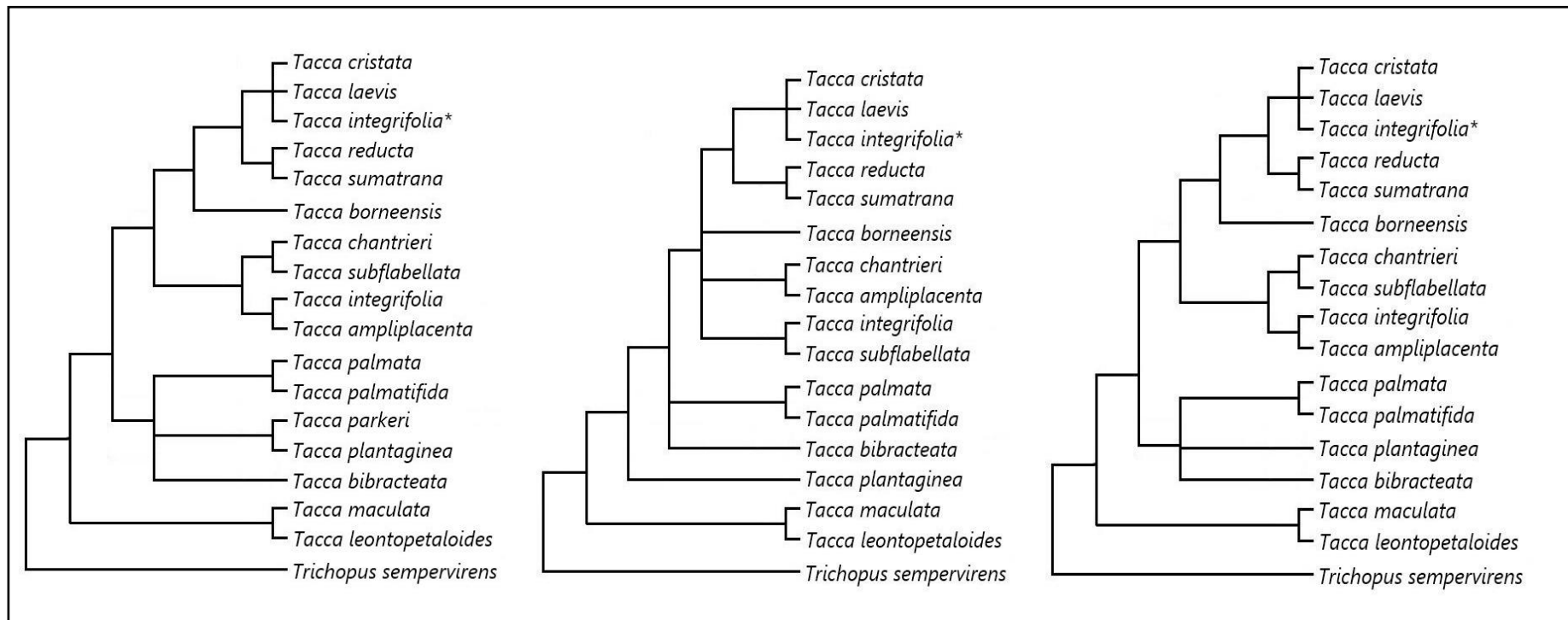


Figure 3.4: Phylogenetic trees from MP analysis only showing the topology of species distribution based on ITS region, matK region and combined data set (From left to right).

Tacca reducta and *T. sumatrana* formed a small distinct clade (BS = 52 – 100, PP = 0.97 – 1.00). These two species had many resemblances in morphologies of its inflorescence. The linear triangular shape of inner bracts was spot characteristic to differentiate these species from the rest. Moreover, other floral characteristics such as bracteoles and flowers are similar in these two species. A *Tacca* sp. (TA102) from Indonesia was also observed with similar inflorescence characteristics, but most of its floral parts (bracts, bracteoles and pedicel) are green colour which is different with dark purple inflorescence of *T. reducta*. Figure 3.5 compared the morphological similarities and differences between *T. reducta*, *T. sumatrana* and TA102.

Whereas, *T. laevis*, *T. cristata*, and South East Asia *T. integrifolia* (in Figure 3.1 – 3.3, “*Tacca cristata*” without accession number) from NCBI database formed a clade with BS value ranged between 58 to 99, and PP = 1.00. Samples with accession number TA46 was found either unresolved or sister to clade with *T. cristata*, *T. laevis*, *T. reducta* and *T. sumatrana*. TA46 develops inflorescence with bracts of combined morphologies of these four species. As shown in Figure 3.5, the inflorescence of TA46 had linear triangular outer bracts, but its inner bracts are spatulate shape. Yet, its short peduncle length is similar to the morphology for *T. reducta*.

Tacca bibracteata which was from Borneo did not show any close relation in the trees with other species from Borneo. But, this species formed a sub-clade with species which had similar inflorescence morphologies such as green, inconspicuous bracts (*T. plantaginea*) and reduced number of ovules (*T. palmata*).



Figure 3.5: Inflorescence of **A:** *T. reducta*; **B:** *T. sumatrana*; **C:** TA102; **D:** TA46.

3.3.2 Ancestral reconstruction based on morphology characters

Measurements on one vegetative character and nine floral structures are listed in Table 3.5. These measurements were converted into character matrices based on the mentioned categories in Table 3.6.

Table 3.5: The data for *T. bibracteata*, *T. borneensis*, *T. cristata*, *T. integrifolia*, *T. laevis*, *T. leontopetaloides*, *T. reducta*, and *T. sumatrana* were measured, whereas data for the rest of *Tacca* spp. were obtained from the descriptions of published reports. Note: “N/A” indicates not applicable and “-” indicates no data recorded.

Species	Leaf shape	Peduncle length (cm)	Inner bracts length (cm)			Inner bracts width (cm)			Inner bract size (cm ²)	Number of inner bract
			Min	Max	Average	Min	Max	Average		
<i>T. amplipectata</i>	entire	40 to 70	10.0	16.0	13.0	8.0	10.0	9.0	117.0	2
<i>T. bibracteata</i>	entire	15 to 23	23.6	28.0	25.8	0.2	0.4	0.3	7.7	2
<i>T. borneensis</i>	entire	34 to 47	7.3	8.6	8.0	7.8	10.0	8.9	71.2	2
<i>T. chantrieri</i>	entire	63	2.5	17.0	9.8	1.5	9.0	5.3	51.9	2
<i>T. cristata</i>	entire	46	15.6	16.0	15.8	6.4	7.0	6.7	105.9	2
<i>T. integrifolia</i>	entire	44	14.2	15.0	14.6	6.8	7.0	6.9	100.7	2
<i>T. laevis</i>	entire	32 to 58	9.8	12.8	11.3	4.0	5.8	4.9	55.4	2
<i>T. leontopetaloides</i>	palmate	91 to 180	4.8	9.8	7.3	1.8	6.0	3.9	28.5	2 to 9
<i>T. palmata</i>	palmate	30 to 80	4.5	10.0	7.3	2.5	6.0	4.3	31.4	2
<i>T. palmatifida</i>	palmate	30 to 60	6.5	12.5	9.5	4.5	7.0	5.8	55.1	2
<i>T. plantaginea</i>	entire	7.5 to 25	0.7	2.5	1.6	0.3	1.0	0.7	1.1	2
<i>T. reducta</i>	entire	21 to 30	4.1	5.2	4.7	1.4	1.8	1.6	7.5	2
<i>T. subflabellata</i>	entire	25 to 30	-	-	6.5	-	-	11.5	74.8	2
<i>T. sumatrana</i>	entire	27	3.8	4.0	3.9	1.8	1.8	1.8	7.0	2
<i>Trichopus sempervirens</i>	entire	-	-	-	-	-	-	-	-	-

Table 3.5 continued

Species	Outer bracts length (cm)			Outer bracts width (cm)			Outer bract size (cm ²)	Colour of bract	Number of bracteoles		
	Min	Max	Average	Min	Max	Average			Min	Max	Average
<i>T. amplioplacenta</i>	6.0	8.0	7.0	3.0	4.0	3.5	24.5	purple	6	26	16
<i>T. bibracteata</i>	3.1	4.4	3.8	2.8	3.6	3.2	12.2	green	10	16	15
<i>T. borneensis</i>	8.2	9.6	8.9	5.6	6.8	6.2	55.2	purple	4	9	6
<i>T. chantrieri</i>	2.0	9.0	5.5	0.8	4.0	2.4	13.2	green/purple	6	26	16
<i>T. cristata</i>	9.8	11.2	10.5	5.8	6.0	5.9	62.0	purple	10	25	15
<i>T. integrifolia</i>	5.5	6.2	5.9	3.5	3.1	3.3	19.5	purple	5	27	16
<i>T. laevis</i>	6.8	9.0	7.9	4.0	6.4	5.2	41.1	purple/white	6	12	9
<i>T. leontopetaloides</i>	4.2	10.9	7.6	2.8	5.0	3.9	29.6	green	9	57	22
<i>T. palmata</i>	2.5	9.5	6.0	2.0	9.0	5.5	33.0	green	N/A	N/A	N/A
<i>T. palmatifida</i>	1.0	2.2	1.6	1.0	1.3	1.2	1.9	green	N/A	N/A	N/A
<i>T. plantaginea</i>	0.9	3.0	2.0	0.5	2.0	1.3	2.6	green	6	20	13
<i>T. reducta</i>	4.2	4.7	4.5	1.1	2.0	1.6	7.2	purple	10	17	14
<i>T. subflabellata</i>	4.0	6.0	5.0	3.0	4.0	3.5	17.5	pink/white	-	-	-
<i>T. sumatrana</i>	4.3	5.0	4.7	1.7	1.8	1.8	8.5	purple		15	11 – 20
<i>Trichopus sempervirens</i>	-	-	-	-	-	-	-	other colour	-	-	-

Table 3.5 continued

Species	Length of bracteoles			Number of flowers			Number of ovules	Reference
	Min	Max	Average	Min	Max	Average		
<i>T. amplipecta</i>	-	40.0	> 22	-	25	11.0 to 20.0	-	Zhang & Li (2008a); Zhang et al. (2011)
<i>T. bibracteata</i>	10.8	25.6	18.2	2	10	3.5	47.3	Drenth (1972)
<i>T. borneensis</i>	13.2	21.0	17.1	8	22	13.6	234.0	-
<i>T. chantrieri</i>	10.0	25.0	17.5	4	25	11.0 to 20.0	285.0	Drenth (1972); Pengklai (1980); Zhang et al. (2005)
<i>T. cristata</i>	10.0	17.1	13.6	4	19	9.9	195.8	-
<i>T. integrifolia</i>	15.5	44.2	29.9	-	30	11.0 to 20.0	351.0	-
<i>T. laevis</i>	11.2	27.8	19.5	3	12	6.6	152.6	-
<i>T. leontopetaloides</i>	19.8	33.2	26.5	7	145	46.8	70.6	Pengklai (1980)
<i>T. palmata</i>	N/A	N/A	N/A	12	23	17.3	8.0	Drenth (1972)
<i>T. palmatifida</i>	N/A	N/A	N/A	-	25	11.0 to 20.0	-	Drenth (1972)
<i>T. plantaginea</i>	-	8.0	≤ 12	1	13	< 10.0	-	Drenth (1972); Hassan & Uddin (2007); Pengklai (1980)
<i>T. reducta</i>	10.1	20.0	15.1	2	9	4.6	109.4	-
<i>T. subflabellata</i>	-	-	-	7	10	< 10.0	-	Ling & Ting (1982)
<i>T. sumatrana</i>	7.1	13.3	10.2	-	4	< 10.0	104.0	-
<i>Trichopus sempervirens</i>	-	-	-	-	-	< 10.0	<40	Sivarajan et al. (1990)

Table 3.6: Character matrices of morphological characters for each species. Leaf shape: 1 = palmate, 2 = entire; Peduncle length: 1 = ≤ 30 cm, 2 = 31 – 60, 3 = > 60 ; Size of inner and outer bracts: 1 = ≤ 20 cm², 2 = 21 – 40 cm², 3 = 41 – 60 cm², 4 = > 60 cm²; Number of inner bracts: 1 = 2, 2 = > 2 ; Inner bract colour: 1 = green, 2 = other colour; Number of bracteoles: 1 = no bracteole, 2 = ≤ 10 , 3 = 11 – 20, 4 = > 20 ; Bracteoles length: 1 = ≤ 12 cm, 2 = 12 – 22, 3 = > 22 cm; Number of flowers: 1 = ≤ 10 , 2 = 11 – 20, 3 = > 20 ; Number of ovules: 1 = ≤ 40 , 2 = 41 – 100, 3 = 101 – 200, 4 = > 200 .

Species	Leaf shape	Length of peduncle	Size of inner bracts	Size of outer bracts	Number of inner bracts	Colour of inner bracts	Number of bracteoles	Length of bracteoles	Number of flowers	Number of ovules
<i>T. amplipecta</i>	2	2	4	2	1	2	3	3	2	0
<i>T. bibracteata</i>	2	1	1	1	1	1	3	2	1	2
<i>T. borneensis</i>	2	2	4	3	1	2	2	2	2	4
<i>T. chantrieri</i>	2	2	3	1	1	2	3	2	2	4
<i>T. cristata</i>	2	2	4	4	1	2	3	2	1	3
<i>T. integrifolia</i>	2	2	4	1	1	2	3	3	2	4
<i>T. laevis</i>	2	2	3	3	1	2	2	2	1	3
<i>T. leontopetaloides</i>	1	3	2	2	2	1	4	3	3	2
<i>T. palmata</i>	1	2	2	2	1	1	1	0	2	1
<i>T. palmatifida</i>	1	2	3	1	1	1	1	0	2	0
<i>T. plantaginea</i>	2	1	1	1	1	1	3	1	1	0
<i>T. reducta</i>	2	1	1	1	1	2	3	2	1	3
<i>T. subflabellata</i>	2	1	4	1	1	2	0	0	1	0
<i>T. sumatrana</i>	2	1	1	1	1	2	3	1	1	3
<i>Trichopus sempervirens</i>	2	0	0	0	0	2	0	0	1	1

Figure 3.6 showed all the trees produced from the morphological mapping. Different colour on branch indicated the differences of character matrices. Tree number 1 was mapped by leaf shape character. It is clear that the ancestral state of leaf shape in genus *Tacca* was palmate shape, species in advance clade were all with entire leaf margin. Fossils of *T. umerii* A.D.Pan, B.F.Jacobs, & E.D.Currano which was dated as Early Miocene (21.7 Ma) revealed the leaves with palmate lobes (Pan et al., 2013). Hence, the ancestral state for palmate leaf shape of genus *Tacca* was further justified. Tree number 2 showed the changes in peduncle length among species across the phylogenetic tree. As indicated, the basal clade species, *T. leontopetaloides*, has the longest peduncle, and the length of peduncle shortened in other species. Thus, the evolution direction of peduncle length was moving from tall inflorescence to much shorter inflorescence.

The evolutionary pattern of inflorescence inner and outer bracts areas did not have a fixed trend. Tree number 3 depicted the reconstruction of inner bracts size traits. As shown by tree number 3, the inner bracts were generally larger in derived clade species. This result was congruent with previously reported finding from Zhang et al. (2011). As predicted, *T. bibracteata* joined *T. plantaginea* near basal clade in which both had inconspicuous inner bracts. However, the inclusion of *T. reducta* and *T. sumatrana* with rather linear inner bracts showed a reversal in the evolutionary direction of inflorescence traits. *Tacca reducta* and *T. sumatrana* which were found in the derived clade exhibit a drastic reduction in inner bract size. Yet, other species (*T. chantrieri* and *T. laevis*) also had a slightly smaller area of inner bracts compared to the rest of the species within the derived clade. In tree number 4, contrasting with inner bracts, most species had a reduced size of outer bracts as compared to the ancestral state supposedly similar to *T. leontopetaloides*. But, an increase in the size of outer bracts was noted in *T. borneensis*, *T. laevis* and *T. cristata*. The size of outer bracts

shrunk again in the derived clade as observed in *T. reducta* and *T. sumatrana*. Although the overall size of inner and outer bracts were bigger in derived clade, some species in that clade had evolved to develop inconspicuous bracts.

Other features related to bracts are the number of inner bracts and colour of bracts. Both of these mapped characters clarified the phylogenetic tree with distinct direction (Tree number 5 & 6). Among the studied species, only *T. leontopetaloides* commonly had inflorescence with more than two inner bracts. This species can develop up to 10 inner bracts with average ranged between six or eight inner bracts. Whereas, the rest of the species normally only have two inner bracts, although inflorescences of *T. laevis* were occasionally observed with three inner bracts in rare cases. For the colour of bracts (Tree number 6), green bracts were assumed to be the ancestral trait whereas other colours such as dark purple and white developed by species in the derived clade.

Next, tree number 7 and 8 indicated the phylogenetic reconstruction for the quantity of bracteoles and the average length of bracteoles, respectively. Generally, *Tacca* spp. encountered loss in the number and length of bracteoles based on the evolutionary trend. The phylogenetic tree indicated that the genus' ancestral state had longer bracteoles in greater amount. The basal clade species, *T. leontopetaloides*, had inflorescence with numerous bracteoles and length more than 22 cm which is more and longer than almost all species in the genus. Bracteole was absent in *T. palmata* and *T. palmatifida*. In addition, the number of bracteoles was reduced in *T. borneensis* and *T. laevis* from Borneo. Besides, *T. plantaginea* and *T. sumatrana* had the shortest bracteoles. However, an exceptional case was observed in *T. amplipecta* and *T. integrifolia* in which the inflorescence develop long bracteoles.

The subsequent tree (tree number 9) were reconstructed using the character of the number of flowers. As shown, the amount of flowers developed by each species shows a clear reduce across the phylogenetic tree. This modification was probably an approach for decreasing geitonogamy. Furthermore, the decrease in flower number also limits the number of anthetic flower to one or two flowers per inflorescence per day. Hence, the chance for inbreeding was minimized. The reduction in bracteoles and flowers maybe a trade-off for other floral traits which are more likely to improve reproductive success. Empirical field observation is required for exceptional species such as *T. amplipecta* to determine whether bracteoles serve any significant role in pollination. For other species, resource for the reproductive function was probably allocated to colour or size of inner bracts or ovule number (refer below) instead of the development of bracteoles and flowers.

The last tree was mapped with the number of ovules, but it wasn't complete with data of all ingroup species. Despite the partially available data, the reconstruction showed an overall increase in the ovules amount. Only *T. palmata* was found to be having the number of ovules less than the ancestral state. *Tacca bibracteata* had a similar range of an average number of ovules (41 – 100) as *T. leontopetaloides*. There was a slight decline in ovules number at the clade with *T. cristata*, *T. laevis*, *T. reducta* and *T. sumatrana*, but still higher than the assumed ancestral state.

“Bet hedging” concept is an evolutionary hypothesis on explaining the development of supplementary strategies (e.g. seed dormancy) as insurance and wager to create reproductive variation under the risk of the stochastic environment (Seger & Brockmann, 1987). Development of ovule overproduction is a bet-hedging strategy on the sporadic deposition of a large amount of pollen (Burd, 1995). This scenario was also termed as parental optimism, predicted the evolution of flowering plants that produce extra ovules for rising fertilization

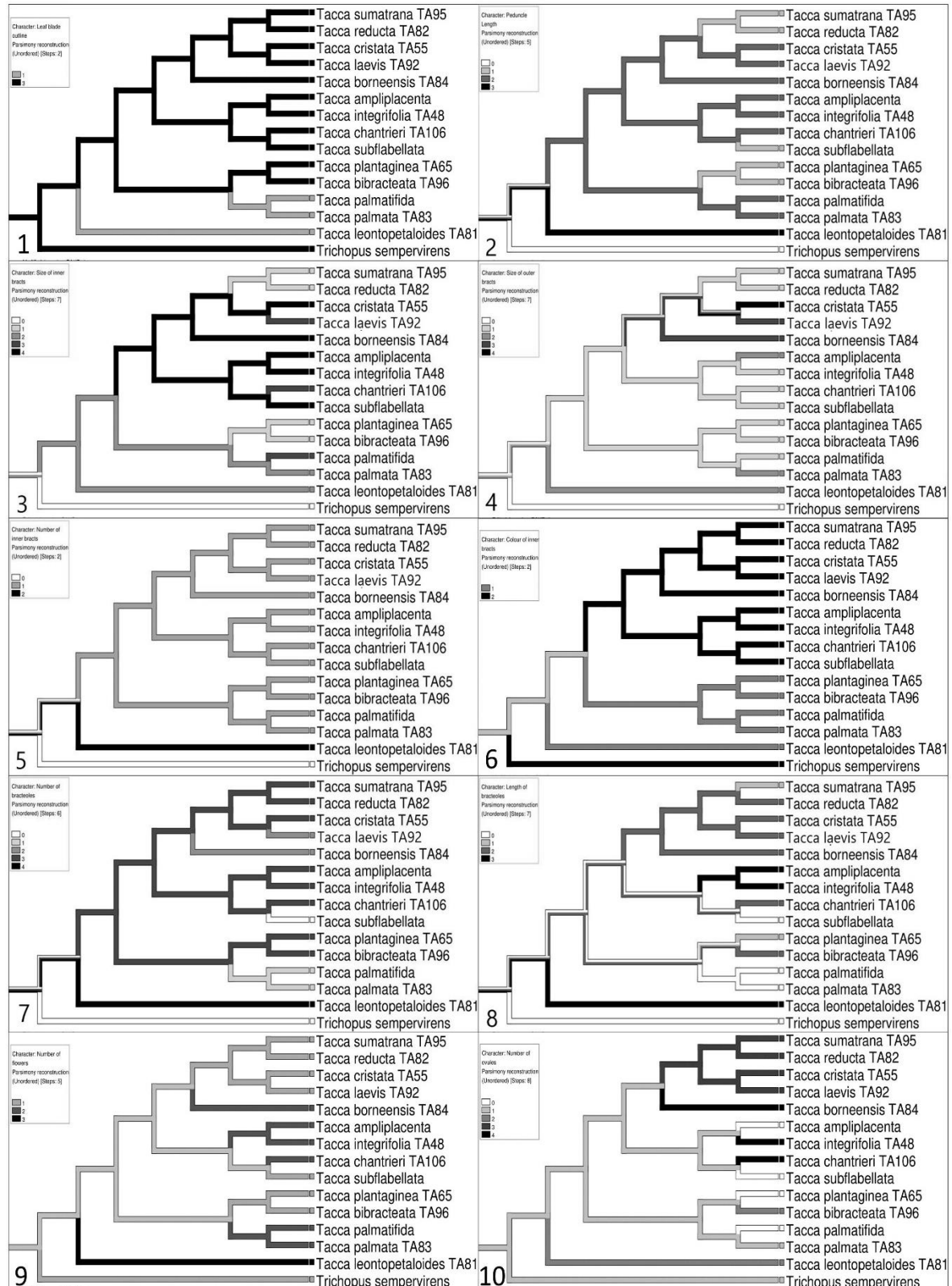


Figure 3.6: Morphology mapping on MP tree from combined dataset. 1: Leaf shape; 2: Peduncle length; 3: Size of inner bracts; 4: Size of outer bracts; 5: Number of inner bracts; 6: Colour of inner bracts; 7: Number of bracteoles; 8: Length of bracteoles; 9: Number of flowers; 10: Number of ovules.

success if pollination occurred more frequently than usual (Mock & Forbes, 1995). This adaptation was observed in the evolution direction of *Tacca* spp. which have higher ovules number per flower, yet empirical observation proved the pollinator visitation was stochastic (Lim & Raguso, 2017).

Recent studies reported that the number of ovules per flower was positively correlated with the degree of pollen limitation among species, and other floral traits such as anther number, flower mass, and corolla length (Holland & Chamberlain, 2007; Knight et al., 2005). A similar trend was shown in tree number 3 and 10, in which most species had the increment pattern in size of inner bracts corresponded with the number of ovules. Selection on larger inner bracts may indirectly increase the production of ovule quantity, or vice versa. Moreover, the ovule number was expected to be higher in plant which relies on specialized pollinators and/or occurs in low population density (Burd, 2009). The number of ovules was reported to be one of the main influence on the seed set variation in flowers (Strelin & Aizen, 2018). Thus, the ovule number's role in plant reproduction was crucial in determining the maximum seed set capacity, and then the size of the next-generation population.

This study included more species and extra morphological characters than the previous study (Zhang et al., 2011). In Zhang et al. (2011), the floral traits of genus *Tacca* were concluded to be evolving from small bracts and short bracteoles to conspicuous bracts and long bracteoles. The inclusion of Bornean *Tacca* spp. in phylogenetic analyses challenged the previous statement. It is because the size of bracts reduced again in the derived clade from *T. borneensis* to *T. laevis* and *T. reducta*. The type of bracts, either inner or outer pair, wasn't specified in previous ancestral reconstruction using morphological characters.

For outer bract's size, most species did not follow the enlarging development trend as mentioned in Zhang et al. (2011). The pair of outer bracts cover all other floral parts, especially the flower buds, throughout the early development of inflorescence. Hence, its function is probably to protect the developing flower buds from exterior threats such as florivore and/or atmospheric conditions. During anthesis, both of these bracts located at unnoticeable position (behind and below other floral structures) on the inflorescence. Thus, the likelihood and efficiency of the outer bracts pair being attractive to pollinators were insignificant, so the size of the outer bract is not crucial for reproductive purposes.

Whereas for inner bract's size, the position of *T. reducta* and *T. sumatrana* on the derived clade denied the hypotheses of Zhang et al. (2011). The reduction in the area of bracts maybe a mechanism of a trade-off between other floral traits, such as developing a higher quantity of bracteoles. Yet, this trade-off remained questionable as both of these structures were supposed to be performing a similar function in luring pollinators. Only the colour intensity of bracts showed the same pattern as reported in Zhang et al. (2011).

In conclusion, phylogenetic reconstruction of genus *Tacca* by using a combination of nuclear ITS and plastid matK regions was sufficient to analyse the relationship at specific level. Most branch lengths of different phylogenetic trees were noticeably short, indicating the limit of resolution of these two DNA regions and the relatively recent radiation, in this case, the floral structures (Wilkin et al., 2005). Ancestral reconstruction according to morphological characters indicated that *Tacca* leaf shape evolved from palmate to entire; peduncle from long to short; outer involucral bracts' size reduced; inner involucral bracts quantity decreased and size initially expanding, later reduced; bracteoles slightly decreased in number and length; the number of flowers decreased, whereas the number of ovules increased.

CHAPTER 4

FLORAL BIOLOGY OF *Tacca* IN SARAWAK

4.1 Introduction

Inflorescences of some *Tacca* developed showy bracts and bracteoles. Unquestionably, these floral structures require high resource investment, but their exact functions were not confirmed (Zhang et al., 2007). Hence, to understand the floral phenology, field observations on *Tacca* species were conducted in their natural habitats. Visiting rate of insect visitors and their visiting hours, as well as their movements, were recorded. Besides, pollen and ovules of *T. borneensis*, *T. laevis*, and *T. reducta* were calculated to obtain the pollen: ovule (P:O) ratio. Field manipulation experiment and controlled pollination experiment were conducted. Field manipulation experiment involved the removal of bracts and bracteoles from inflorescence to determine whether or not their absence had an impact on fruit and seed set. Controlled pollination experiment included works on artificial self-pollination and artificial cross-pollination. In this experiment, the number of seeds of each species were analysed and compared between control and treatments. This chapter's main objective was to understand the flowering biology of *Tacca* spp. in Sarawak. The aim of the experimental tests was to examine whether investigated *Tacca* spp. are dependent on their conspicuous bracts and bracteoles for increasing probability of pollination success. In addition, the capability of *Tacca* spp. on self-compatibility was investigated as well.

4.2 Materials and methods

4.2.1 Plant and study area

Within Sarawak, *T. bibracteata*, *T. borneensis*, *T. laevis*, *T. leontopetaloides*, *T. palmata*, and *T. reducta* were included in this floral study (Table 4.1). *Tacca cristata* from Peninsular Malaysia was also added as an outgroup for comparison. Observations for floral biology of *Tacca* spp. were conducted from February 2017 until February 2019.

Table 4.1: *Tacca* species and the locality of their type specimen.

Species	Locality of type	Reference
<i>T. bibracteata</i>	Sarawak Long Kapa, Mount Dulit (Ulu Tinjar) IVth Division	http://specimens.kew.org/herbarium/K000292187
<i>T. borneensis</i>	Matang, Kuching	Boyce & Julia (2006)
<i>T. cristata</i>	Singapore	http://specimens.kew.org/herbarium/K000292197
<i>T. laevis</i>	Sylhet, Bangladesh	http://specimens.kew.org/herbarium/K001120041
<i>T. leontopetaloides</i>	Tahiti	http://specimens.kew.org/herbarium/K000958801
<i>T. palmata</i>	Java, Indonesia	Drenth (1972)
<i>T. reducta</i>	Bau, Kuching	Boyce & Julia (2006)

Six study areas were chosen for floral observations and experiments (Figure 4.1). The selected localities were Pantai Sampadi, Lundu (PS), Kubah National Park, Matang (KNP), Kampung Segong, Bau (KS), Mount Singai, Bau (MS), Kampung Peninjau Lama, Bau (KPL), and Ranchan Recreational Park, Serian (RRP) (Table 4.2). The occurrence of different species and population size varied among localities. Species and their respective population size were indicated in Table 4.3. *Tacca* spp. were found co-existing together in its natural habitat; for instances, *T. leontopetaloides* and *T. palmata* in PS, *T. borneensis* and

T. laevis in KNP, *T. borneensis*, *T. palmata* and *T. reducta* in RRP. For *Tacca cristata*, the locality selected was Forest Research Institute Malaysia (FRIM), Selangor (Figure 4.2).

Firstly, *T. leontopetaloides* had about 40 individuals within the population. At PS, *T. palmata* was recorded with about ten individuals. Whereas, *T. palmata* at KPL and RRP had two and five individuals, respectively. Other than *T. palmata*, KPL was found to have one *T. borneensis* and about 20 individuals of *T. reducta*'s population. For RRP, there were three *T. borneensis* and about 140 *T. reducta* within the localities. Populations of *T. borneensis* were also observed at KNP and MS with approximately 15 and 25 individuals, respectively. Seven individuals of *T. laevis* also occurred at KNP. A population of *T. laevis* with about 17 individuals was spotted in KS. Lastly, FRIM, which had *T. cristata* only, had about 85 individuals. *Tacca* spp. with the same localities were considerably distant (PS: >500m; KNP: >1km) from one another except *T. palmata* and *T. reducta*. These two species found to be growing adjacently in both KPL & RRP.



Figure 4.1: Localities for sampling sites, the details of each locality were listed in Table 4.1 (Red rectangular area in top right map indicated the location of sampling sites in Borneo; map was retrieved from Google Earth).

Table 4.2: The localities of sampling sites with their locality code, GPS and altitude of species included.

No.	Localities	Population code	Species	GPS	Elevation (m a.s.l.)
I	Pantai Sampadi, Lundu	PS	<i>T. leontopetaloides</i> & <i>T. palmata</i>	1°41'55.5"N, 110°5'46.9"E	25
II	Kubah National Park, Matang	KNP	<i>T. borneensis</i> & <i>T. laevis</i>	1°36'24.7"N, 110°11'18.8"E	200
III	Kampung Segong, Bau	KS	<i>T. laevis</i>	1°31'12.1"N, 110°8'18.9"E	53
IV	Mount Singai, Bau	MS	<i>T. borneensis</i>	1°30'13.3"N, 110°10'36.1"E	130
V	Kampung Peninjau Lama, Bau	KPL	<i>T. borneensis</i> , <i>T. palmata</i> & <i>T. reducta</i>	1°25'51.4"N, 110°13'32.4"E	130
VI	Ranchan Recreational Park, Serian	RRP	<i>T. borneensis</i> , <i>T. palmata</i> & <i>T. reducta</i>	1°8'34.4"N, 110°34'55.3"E	57
	Forest Research Institute Malaysia	FRIM	<i>T. cristata</i>	3°13'59.1"N, 101°37'53.4"E	83

Table 4.3: Species population size in each sampled localities (“–” represents absence of the species in the localities).

Locality code	Population size					
	<i>T. borneensis</i>	<i>T. laevis</i>	<i>T. leontopetaloides</i>	<i>T. palmata</i>	<i>T. reducta</i>	<i>T. cristata</i>
PS	–	–	40	10	–	–
KNP	15	7	–	–	–	–
KS	–	17	–	–	–	–
MS	25	–	–	–	–	–
KPL	1	–	–	2	20	–
RRP	3	–	–	5	140	–
FRIM	–	–	–	–	–	85

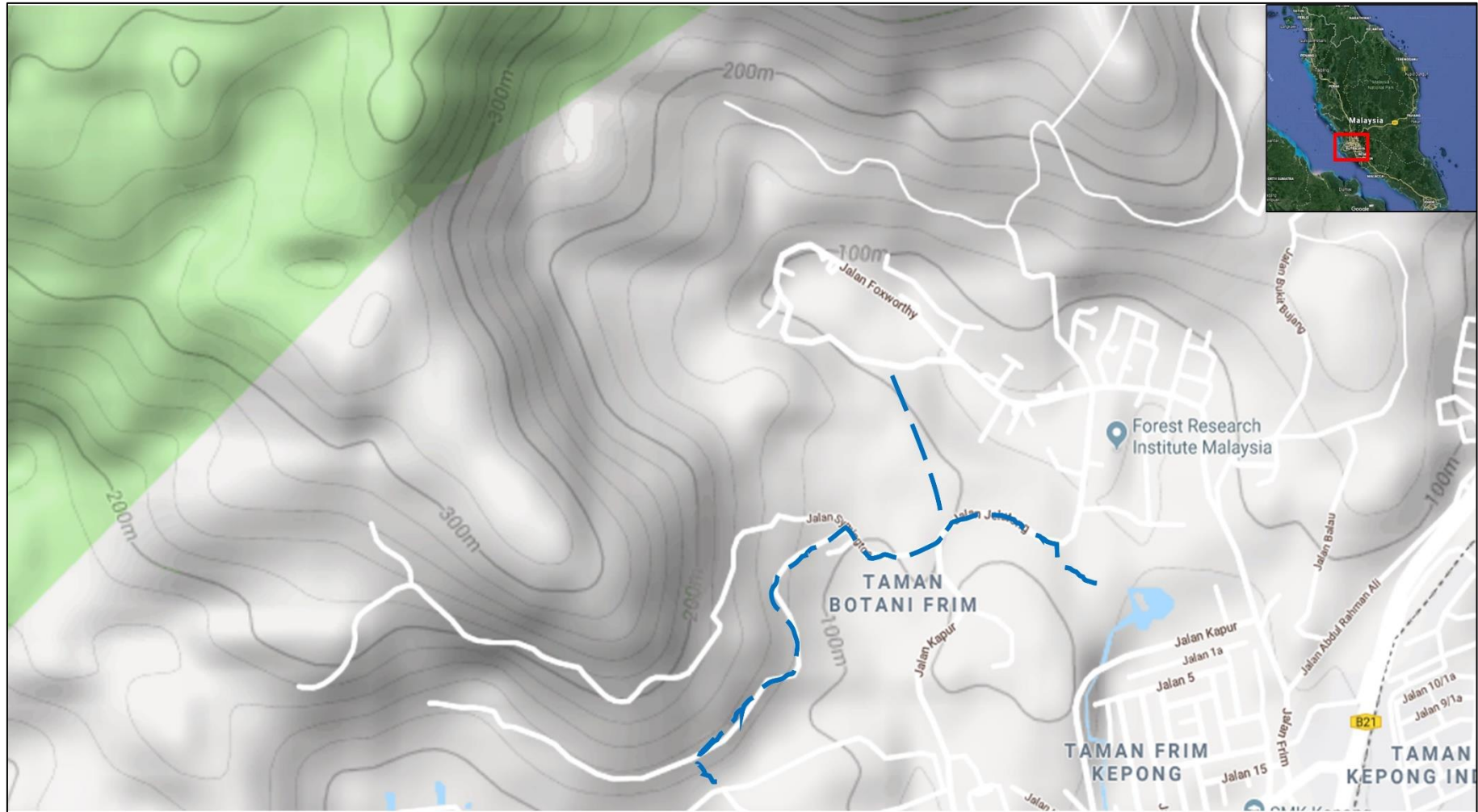


Figure 4.2: Locality for sampling site of *T. cristata*, the details of each locality were listed in Table 4.1 (Blue dash lines indicated the trails with *T. cristata*; red rectangular area in top right map indicated the location of sampling sites in Selangor state; map was retrieved from Google Earth).

4.2.2 Plant description

Plant descriptions of each species were listed as below (modified from Boyce & Julia, 2006 and Drenth, 1976):

4.2.2.1 *Tacca bibracteata* Drenth

Moderate terrestrial **herb** to 40 cm tall. **Stem** rhizomatous, ascending and ultimately epigeal, 2–4 cm thick, clothed with persistent leaf bases. **Leaves** 6–10 together, petioles ascending, 5–19 cm long, c. 2–8 mm diam, D-shaped in overall cross-section, sharply sulcate-canaliculate, bluntly keeled on the dorsal side, pale to mid-green; petiolar sheath c. 1/4–1/2 length of petiole (3.5–9.5 cm long), hyaline and shortly (c. 2mm) ligulate; ligule dolabriform-acute, margins minutely erose; lamina ascending to somewhat spreading, oblong-lanceolate, 16–32.6 x 7–14.4 cm, base acute, apex shortly acuminate, margins smooth, lamina deep glossy green adaxially, paler and less glossy abaxially; mid-rib strongly pronounced abaxially and sunken adaxially; primary lateral veins 4–6 per side, very pronounced abaxially, all arising from the basal half of the mid-rib, interprimary lateral veins absent, secondary veins forming a rather obscure untidy reti-tesellate network and with a weak interprimary collecting vein running through. **Inflorescence** solitary to 2 at different developmental stages per plant; peduncle terete, 20–31 cm tall, pale green lightly to heavily dark purple-mottled, intensifying towards the base; involucre bracts strongly heteromorphic; outer pair dorsoventrally positioned, ovate, 3.1–4.4 x 2.8–3.6 cm, briefly decurrent basally and fused to form a short pocket, mid-green with the primary veins and apical portion stained purple; inner pair laterally positioned, filiform, 23.6–28 cm x 2–4 mm, basally mid-purple fading to pale yellow-green at the tips; filiform bracts 8–15 per inflorescence, very variable

in length with c. 2.5–25.6 cm lengths in one inflorescence, pale green. **Flowers** up to 10 but usually fewer (5 or less) per inflorescence; pedicel triangular in cross-section, 1–3.5 cm long, pale greenish purple; gynoecium obpyramidal, 1–1.8 x 1.1–1.4 cm, strongly 6-ribbed, pale greenish-purple with the ribs dull purple, perianth inserted annularly onto top of gynoecium; outer perianth lobes broadly ovate, 10–14 x 18–23 mm, reflexing at anthesis green purple-stained abaxially, deep velvety purple adaxially; inner perianth lobes ovate, tip notched, 4–5 x 1–12 mm, bright green abaxially, deep velvety purple adaxially. **Infructescence** declinate by twisting of the peduncle base, almost always solitary fruited, fruit subtended by the persistent outer involucral bracts, ovary obpyramidal, deep c. 2.5 x 1.5 cm, dull purple, topped by the persistent outer and basal half of the inner perianth lobes. **Seeds** not observed (Figure 4.3).



Figure 4.3: *Tacca bibracteata*. **A:** Vegetative part and inflorescence with anthetic flower (arrow). **B:** Inflorescence with flower bud (arrow). **C:** Post-anthetic flowers (arrow).

4.2.2.2 *Tacca borneensis* Ridl.

Moderate to robust terrestrial **herb** to 1.2 m. tall. **Stem** rhizomatous, hypogeal, creeping with the active apex ascending, up to 3.5 cm thick, clothed with persistent leaf bases and frequently rooting through these. **Leaves** 13–17 together; petioles ascending, 44.8–59.8 cm long, 12–19 mm diam, subterete in overall cross-section, pronounced deeply canaliculate, sharply 2-keeled on the dorsal side, mid-green, stained deep purple-brown at the base; petiolar sheath c. 1/6–1/5 length of petiole, hyaline; lamina ascending to recurved, oblong-lanceolate to oblong, 46.2–71 x 20.2–29.4 cm, base oblique, broadly ovate to unequally weakly cordate, apex acuminate, margins smooth, lamina glossy mid-to deep green, paler and less glossy abaxially; mid-rib strongly raised abaxially, sunken adaxially, primary lateral veins 9–12 per side, prominent abaxially, sunken adaxially, interprimary lateral veins less prominent than primaries, secondary veins forming a tessellate network with a variably pronounced interprimary collecting vein running through the middle of each trans-interprimary area. **Inflorescence** solitary to up to 3 at different developmental stages per plant; peduncle sub-erect to decumbent and apically ascending at anthesis, 3–5 angled or weakly 3–5 winged, 27.6–47.8 x c. 1.1 cm, mid-green to more-or-less wholly stained purple-brown; involucre bracts homeomorphic; outer pair dorso-ventrally positioned, ovate-triangular, basally briefly clawed, 8.2–9.6 x 5.6–6.8 cm, velvety very deep purple-black, rarely pale purple with deep purple veining, claw paler, occasionally almost white; inner pair laterally positioned, ovate to broadly ovate, clawed basally, 7.3–8.6 x 7.8–10 cm, deep lustrous purple-black, claw paler to almost white; filiform bracts 3–9 per inflorescence, 13.2–21 cm long, deep purple. **Flowers** 8–25 per inflorescence; pedicel triangular in cross-section, 2–3.4 cm long, dark purple, initially erect, later in anthesis pendent; gynoecium obpyramidal, 2–2.2 cm long x 1.2–1.4 cm wide at apex, 6-ribbed, purple with the ribs darker,

perianth inserted annularly onto top of gynoecium; outer perianth lobes rounded, 6–8 x 5–6 mm, reflexing at anthesis, velvety deep purple; inner perianth lobes ovate, 5–8 x 8–9 mm, deep velvety purple. **Infructescence** prostrate by twisting of the peduncle base, many-fruited, involucre bracts marcescent well prior to fruit maturation; fruits obpyramidal, c. 3.5 x 1.5 cm, semi-glossy deep purple. **Seeds** weakly laterally compressed-reniform, c. 3.5 x 1.5–2 mm, pale brown (Figure 4.4).



Figure 4.4: *Tacca borneensis*. **A:** Whole plant without inflorescence. **B:** Inflorescence. **C:** Fruits.

4.2.2.3 *Tacca laevis* Roxb.

Slender to moderately robust terrestrial **herb** to 73 cm tall. **Stem** rhizomatous, hypogeal, creeping with the active apex ascending (W Sarawak) or epigeal (NE Sarawak), up to 2.6 cm thick, clothed with persistent leaf bases and frequently rooting through these. **Leaves** 5–22 together, petioles ascending, 4.4–18 cm long, 9–12 mm diam, D-shaped in overall cross-section, canaliculate, 2-keeled on the dorsal side, pale to mid-green sometimes stained purple at the base; petiolar sheath c. 1/5 length of petiole, hyaline; lamina thinly to rather thickly coriaceous, ascending, lanceolate, 40.6–67.4 x 8.8–12.5 cm, base acute, decurrent, c. 5 cm along petiole, apex acute to acuminate, margins smooth, lamina pale to mid-green, weakly glossy adaxially, paler and less glossy abaxially; mid-rib prominently raised abaxially, sunken adaxially, primary lateral veins 10–11 per side, slightly sunken adaxially, interprimary lateral veins slightly less prominent than primaries, secondary veins forming a very obscure network with an obscure interprimary collecting vein running through the middle of each trans-interprimary tessellate area. **Inflorescence** solitary to 3 at different developmental stages per plant; peduncle erect, weakly 3 angled, 32.8–67 cm tall, pale green stained deep purple especially near the base; involucral bracts heteromorphic: outer pair dorso-ventrally positioned, narrowly ovate, 6.8–9 x 4–6.4 cm, deep purple-black to pale lavender purple, rarely white with lilac veining; inner pair laterally positioned but ascending and ultimately sub-erect at anthesis, spatulate, 9.8–12.8 x 4–5.8 cm, pale lavender purple, rarely white with lilac veining; filiform bracts 6–12 per inflorescence, 7.6–27.8 cm long, pale to mid- or deep purple basally, fading to white at the tip. **Flowers** 3–12 per inflorescence; pedicel triangular in cross-section, 0.8 cm long, pale to mid-purple, initially erect, later in anthesis reflexing, thence pendent; gynoecium widely obpyramidal, c. 1.4 cm long x c. 1.3

cm wide at apex, 6-ribbed, greenish-purple to dark purple, the ribs darker purple, perianth inserted annularly onto top of gynoecium. Outer perianth lobes oblong, rounded 10–17 x 5–7 mm, reflexing at anthesis, dark purple; inner perianth lobes oblong, 12–17 x 6–9 mm, deep velvety purple on both surfaces. **Infructescence** declinate by twisting of the peduncle base, few to many-fruited, involucre bracts marcescent well prior to fruit maturation; fruit obpyramidal, light glossy green 4.8–6 x 2.4–3.2 cm. **Seeds** weakly laterally compressed-reniform, 5 x 3 mm, dark brown, 6-ribbed (Figure 4.5).



Figure 4.5: *Tacca laevis*. **A:** Whole plant without inflorescence. **B:** Inflorescence. **C:** Fruits.

4.2.2.4 *Tacca leontopetaloides* (L.) Kuntze

Tuber depressed-globose or broadly ellipsoid, smooth, 1.5–5 cm high x 1–8 x 0.5–4 cm diam, white when young, older dark grey to brown, white within, provided with an apical cavity emitting the leaves and inflorescences, replacing annually with a new tuber which arises from a downward-growing runner-like thick rhizome at a lower level and remains dormant after yearly death of aerial parts of the original plant. **Cataphyll** linear-lanceolate, 8–21 x 1.2–3 cm. **Leaves** 1–3, broadly obovate, ovate, or oblong-ovate in outline, palmately 3-sect, each of the 3 segments pinnately lobed to dissected, 53–131 x 59–171 cm; lobes orbicular to linear; petiole hollow, 30–87.5 x 1–3.5 cm, sheath 2–25 x 0.6–3.5 cm. **Inflorescences** solitary; peduncle hollow, green, 91.2–180 x 1.1–3 cm; involucral bracts heteromorphic, light to dark green, sometimes with fine purplish margin; outer pair dorso-ventrally positioned, sessile, (ob)ovate, oblong, or lanceolate, attenuate or cuneate base, acuminate at the apex, acumen entire or 2–3 dentate, 4.2–10.9 x 2.8–5 cm, green; inner pair 2–9, laterally positioned but ascending and ultimately sub-erect at anthesis, spatulate, acuminate at the apex, curvinerved with pinnate side nerves, 4.8–9.8 x 1.8–6 cm; the small bracts up to 4 but usually absent, linear-lanceolate, sessile, with acute apex, 6.5–17.8 x 1.1–2.8 cm; filiform bracts 9–57, 8–33.2 cm, pale green basally, (dark) purple or dark blackish-brown to the tip. **Flowers** 14–145 per inflorescence; pedicel 2–6.6 cm x 1 mm, yellowish green; gynoecium 6–22 x 4–9 mm; perianth lobes mostly fleshy with membranous margins, slightly reflex during anthesis, persistent, outer perianth lobes elliptic or ovate (lanceolate), (1.5–) 10–12 x 2 mm, green purple-stained at tip, inner perianth lobes (broadly) ovate or oblong-ovate, 10–12 x 4 mm, green fading to white at tip. **Infructescence** persists erect position while maturing, almost always solitary fruited, fruit mostly globose, sometimes ellipsoid or ovoid, subtended by the persistent involucral bracts and bracteoles, 2.6–5 x 1.4–3.2 cm, pendulous, pale to

darker green, finally pale orange; pericarp up to 1.5 mm thick. **Seeds** ovoid to ellipsoid, flattened, 6.5–9 x 4–5 x 2–5 mm glabrous, yellowish brown, with a spongy white testa, 14–19-ribbed (Figure 4.6).

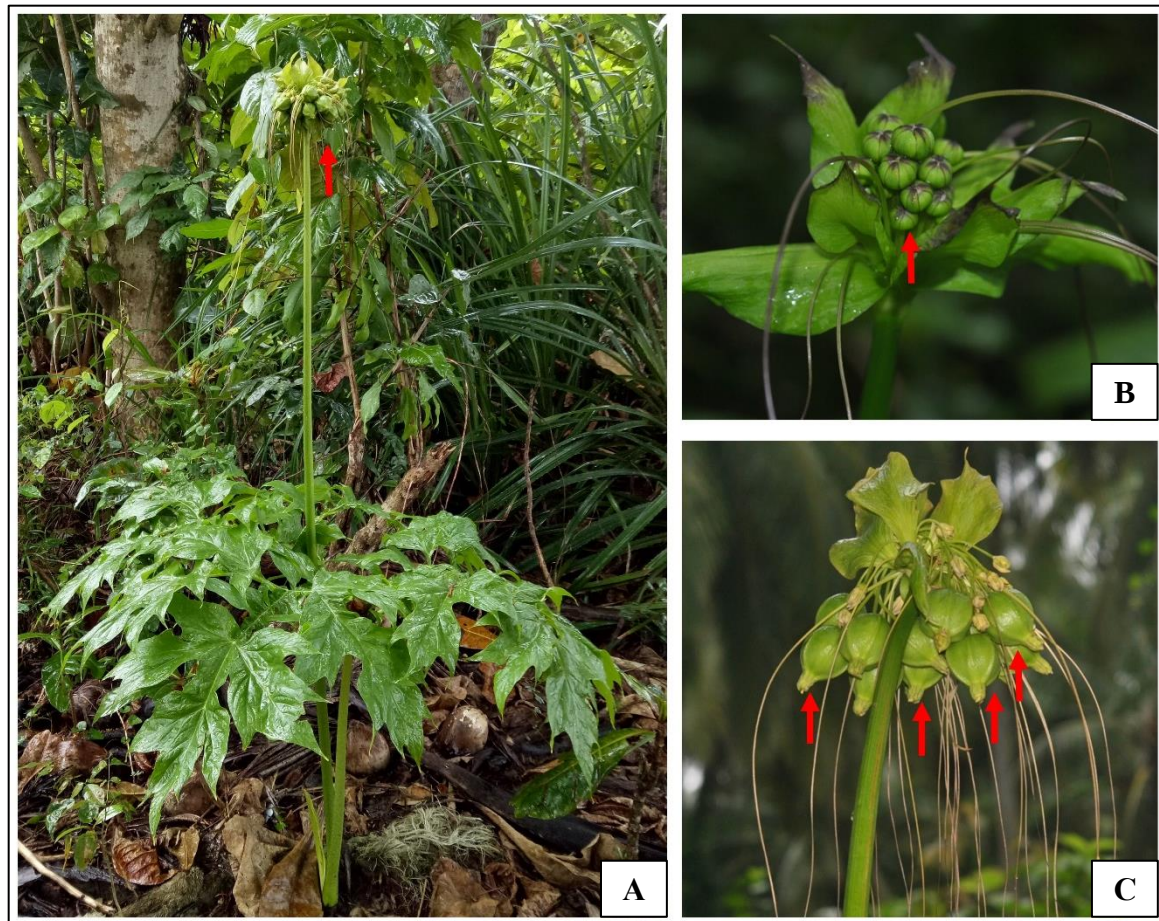


Figure 4.6: *Tacca leontopetaloides*. **A:** Whole plant with infructescence (arrow). **B:** Inflorescence (arrow indicates flowers). **C:** Fruits (arrows).

4.2.2.5 *Tacca palmata* Blume

Tuber globose to broadly ellipsoid, 1–2.5 cm high x 1.5–5 x 1.3–3 cm, fleshy, light brown with an apical cavity from which the leaves and inflorescences emerge. **Leaves** 1–3 (–5), broadly reniform or semi-orbicular in outline, 3–13, usually 4–8-palmatipartite, 7–36 x 7.5–40 cm; base attenuate; lobes (narrowly) obovate, elliptic, or (linear) lanceolate, 6–25 x (0.5–) 1–10 cm, with attenuate base and acuminate apex, the outer lobes mostly smaller than the inner ones; petiole (12–) 15–60 (–75) x 0.1–0.4 cm, sheath 2.5–7.5 x 0.3–0.8 cm. **Inflorescences** 1 or 2 (or 3); peduncle 20–80 x 0.2–0.5 cm; involucral bracts homomorphic, decussate, green tinged with violet; outer pair dorso-ventrally positioned, (broadly) ovate, 2.5–9.5 x 2–9 cm, sessile, apex acuminate; inner pair laterally positioned, ascending, broadly ovate or cordate, 4.5–10 x 2.5–6 cm, with indexed margins in the basal part, base attenuate, apex acuminate, sometimes caudate. **Flowers** up to 30-flowered per inflorescence, green, tinged violet brown, brown violet, or dark violet; pedicel 10–20 x 0.5–1 mm, light green; gynoecium 2–5 x 4–8 mm; outer perianth lobes (broadly) ovate, rarely elliptic, 2–6 x 2.5–6 mm, obtuse or rounded at the apex; inner perianth lobes acuminate apex, 3–5 x 2–4 mm, indexed; perianth lobes curve inward when anthesis end, covering the flower. **Infructescence** persists erect position while maturing, almost always solitary fruited, fruit globose, up to 1 cm, subtended by the persistent involucral bracts, mostly with 3 distinct and 3 indistinct ribs, green, yellow, orange and finally bright red when mature, pericarp up to 1 mm thick. **Seeds** up to 11 in each fruit, more or less pyramidal with a rounded base, 3–5 x 2–4 x 2–3 mm, 15–20-ribbed (Figure 4.7).

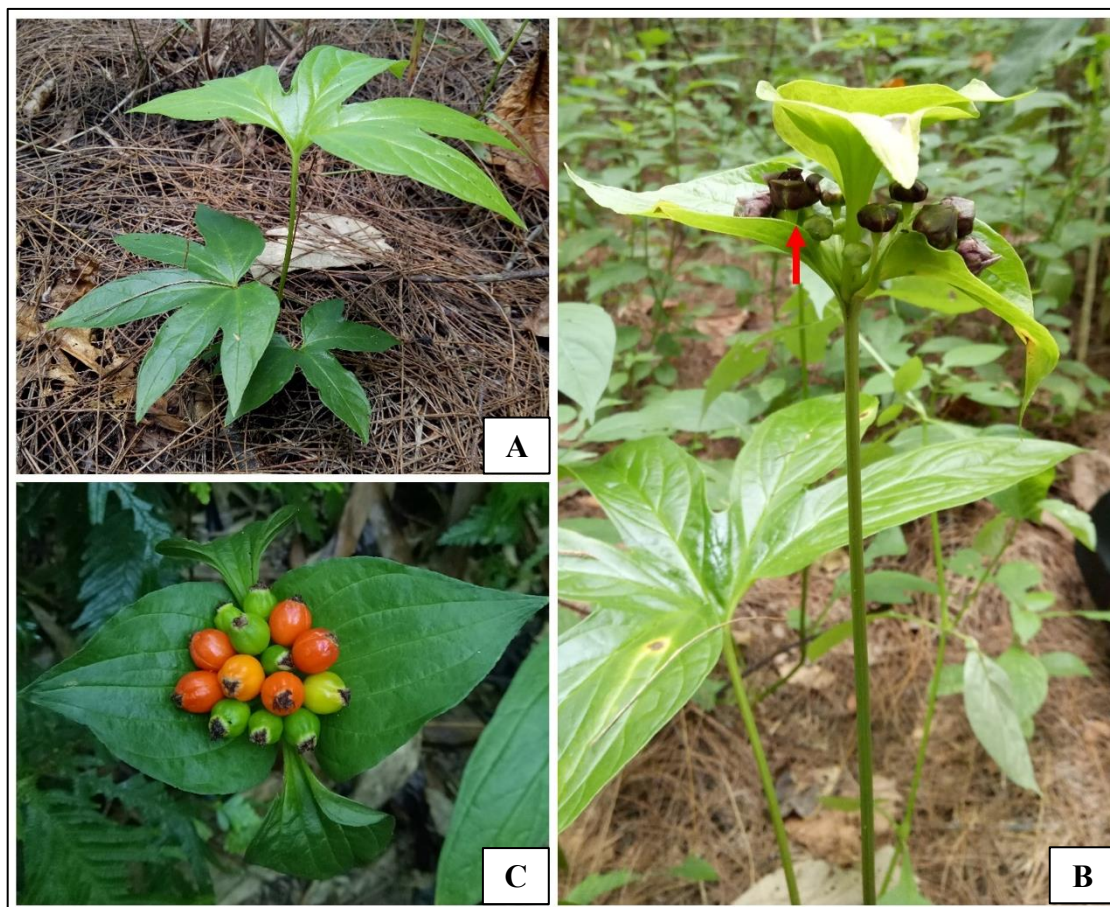


Figure 4.7: *Tacca palmata*. **A:** Whole plant without inflorescence. **B:** Inflorescence (Arrow indicates flowers). **C:** Fruits.

4.2.2.6 *Tacca reducta* P.C. Boyce & S. Julia

Slender to moderately robust terrestrial **herb** to 50 cm tall. **Stem** rhizomatous, hypogeal, creeping with the active apex ascending, 1.5–2 cm thick, clothed with persistent leaf bases and frequently rooting through these. **Leaves** 7–10 together; petioles ascending, up to 6–12 cm long, 2–3 mm diam, D-shaped in overall cross-section, shallowly to rather pronounced canaliculate, sharply 2-keeled on the dorsal side, mid-green variously speckled and stained deep purple-brown notably at the base and along the dorsal keels; petiolar sheath c. 1/5 length of petiole, hyaline; lamina ascending to recurved, lanceolate, 19.8–37.4 x 6–10.2 cm, base acute, decurrent c. 3 cm along petiole, apex acute to acuminate, margins smooth to very

slightly crenulate-dentate, lamina mid-green, slightly glossy adaxially, paler and less glossy abaxially; midrib strongly subterete to raised abaxially, sunken adaxially, primary lateral veins 8–10 per side, slightly sunken adaxially, interprimary lateral veins much less prominent than primaries, secondary veins forming a rather prominent tessellate network with an obscure interprimary collecting vein running through the middle of each trans-interprimary tessellate area. **Inflorescence** solitary to 3 at different developmental stages per plant; peduncle terete to weakly 3–5 angled, 21–36.2 cm tall, pale green more-or-less wholly stained and speckled deep purple-brown; involucre bract homeomorphic: outer pair dorso-ventrally positioned, linear-triangular to linear-ovate, 4.2–4.7 cm x 1.1–2 cm, slightly lustrous very deep purple-black; inner pair laterally positioned, linear-triangular to linear-ovate, 4.1–5.2 cm x 1.4–1.8 cm, deep lustrous purple-black; filiform bracts 8–18 per inflorescence, very variable in length with 10.1–20 cm long, deep purple basally, fading to pale green at the tip. **Flowers** 2–9 per inflorescence; pedicel triangular in cross-section, 1.5–2.5 cm long, mid-purple, initially erect, later in anthesis reflexing, thence pendent; gynoecium broadly obpyramidal, 1.6–1.7 cm long x 1.2–1.3 cm wide at apex, strongly 6-ribbed, dull greenish-purple with the ribs darker purple, perianth inserted annularly onto top of gynoecium; outer perianth lobes oblong, rounded with a brief acumen, 13–16 x 9–10.5 mm, reflexing at anthesis, purple with a darker purple reticulations, deep velvety purple adaxially; inner perianth lobes ovate, 12–15 x 12–14 mm, deep velvety purple on both surfaces. **Infructescence** declinate by twisting of the peduncle base, few-fruited, involucre bracts marcescent well-prior to fruit maturation; fruit obpyramidal, deep slightly glossy purple 3–4.3 x 2.3–2.7 cm, dull purple. **Seeds** weakly laterally compressed-reniform, c. 3.5 x 1.5–2 mm, pale brown (Figure 4.8).

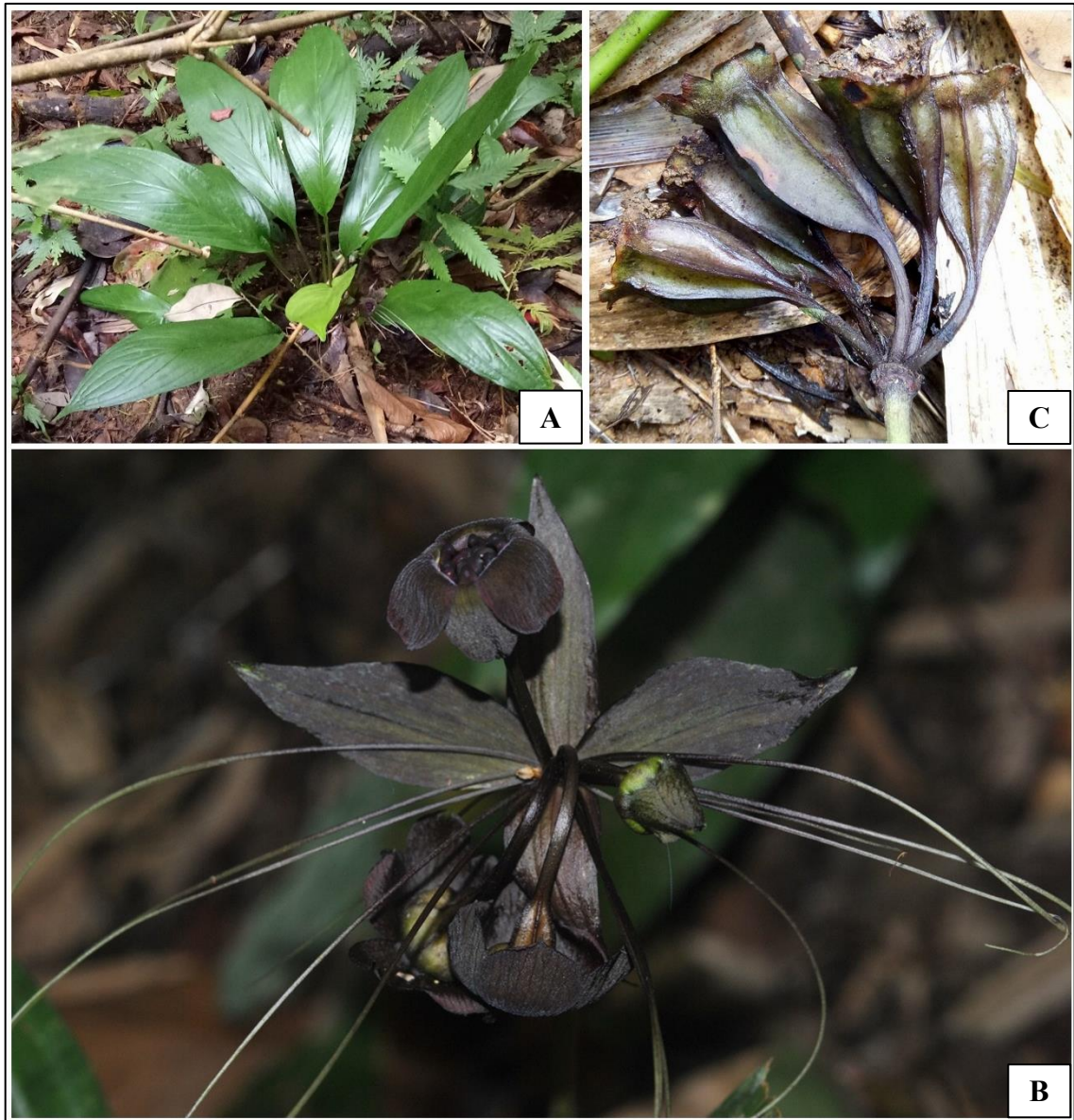


Figure 4.8: *Tacca reducta*. **A:** Whole plant without inflorescence. **B:** Inflorescence. **C:** Fruits.

4.2.2.7 *Tacca cristata* Jack

Slender to moderately robust terrestrial **herb** to 1 m tall. **Stem** up to 12 x 3 cm. **Leaves** 2–13, rosulate, very variable, usually oblong(-ovate) or lanceolate, more rarely elliptic, oblong-obovate or linear-lanceolate, greyish green, 40–65 x 3–24 cm, base attenuate, rarely cuneate or rounded, apex acuminate; nerves pinnate; petiole 4.5–41 cm x 2–6 mm, sheath 2.5–17 x 0.5–1.5 cm. **Inflorescences** 1–4 (–5); peduncle 46.2–65 (–100) cm x c. 12 mm, dark violet, blackish purple, red, or rarely brown; involucral bracts heteromorphic, outer pair dorso-ventrally positioned, opposite, sessile, elliptic, oblong, or (ovate) lanceolate, 9.8–14 x c. 6 cm, green to purple, veined deep purple, apex acute, acuminate, rarely cuspidate; inner pair laterally positioned but ascending and ultimately sub-erect at anthesis, sessile or with attenuate to cuneate base, (ob)ovate, oblong-(ob)ovate, (ob)-lanceolate, or spatulate, rarely orbicular, c. 15 x c. 7 cm, white, shaded purple, veined black, apex acuminate or cuspidate; filiform bracts 10–25, up to 17 cm x 0.2–1 mm, white or bright yellow green, on base darker. **Flowers** up to 30-flowered per inflorescence, c. 2 x 1.5 cm; buds pale greenish, flowers green, greenish-violet, brownish-purple, or blackish-violet, the colour becomes steadily darker; pedicel c. 3 cm x c. 3 mm, dark red or blackish-purple; gynoecium 2.1–2.3 x 15–16 mm; perianth lobes mostly reflexed during anthesis and caducous; outer perianth lobes elliptic, triangular or oblong, c. 20 x 10 mm; inner perianth lobes broadly obovate or broadly ovate, c. 20 x 16 mm. **Infructescence** declinate by twisting of the peduncle base, fruit few to many-fruited, involucral bracts marcescent well prior to fruit maturation; fruit obpyramidal, up to 5 x 2.5 cm, green to black, tinged with purple, pericarp up to 2 mm thick. **Seeds** ovoid convex-concave, 3.5–6 x 1–3.5 x 1–2 mm, glabrous to strongly papillose, 6–16-ribbed (Figure 4.9).



Figure 4.9: *Tacca cristata*. **A:** Whole plant without inflorescence. **B:** Inflorescence. **C:** Fruits.

4.2.3 Floral biology and floral visitors

The observation period and the number of inflorescences in each locality were indicated in Table 4.4. Field works on *T. cristata* were conducted in the interval of every two months. One week observation was made before the inflorescences' anthesis began. Any changes on the inflorescence bud were recorded. During anthesis, observations were made every hour until the end of anthesis. Any distinctive movements or activities of the inflorescence such as anthetic phases, inflorescence structures movement, floral scent emission, and pollen extrusion were recorded as well.

Table 4.4: Observation period and the number of inflorescences replicate for each species on flowering phenology (localities in parentheses).

Species	Observation period	Number of inflorescences observed
<i>T. borneensis</i>	April 2017 – June 2018	3 (KNP), 2 (MS), 1 (RRP)
<i>T. laevis</i>	June 2017 – June 2018	2 (KNP), 3 (KS)
<i>T. leontopetaloides</i>	September 2017	1 (PS)
<i>T. palmata</i>	August 2017 – January 2018	2 (PS), 2 (RRP)
<i>T. reducta</i>	March 2017 – March 2018	8 (RRP)
<i>T. cristata</i>	March 2017 – March 2018	5 (FRIM)

For each inflorescence, the number of flowers and number of bracteoles were recorded. In addition, the data on the number of anthetic flowers per inflorescence each day throughout the observation period was also included.

Other than field observations, 9 – 18 flowers from each species (for *T. palmata* only two flowers) were collected to estimate their ovule number. For pollen estimation, at least five flowers of each species (*T. borneensis*, *T. cristata*, *T. laevis* & *T. reducta*) were collected. Pollen was not counted for *T. palmata* due to low sample availability. All anthers in a flower were removed and immersed into 500 μ L of ethanol. Whereas, the rest of the flower part was dissected for ovules count. Anthers which were immersed in ethanol were vortexed to allow pollen distribute evenly in the ethanol. Then, 10 μ L of the solution was pipetted and divided into 10 droplets on a glass slide. This step was repeated for another four times, and a total of 50 μ L were pipetted from the solution. The amount of pollen present in each droplet

was observed and counted under a dissecting microscope. The total number of pollen in the 50 droplets was used to approximately estimate the total number of pollen in the flower.

$$\text{Estimated total no. of pollen} = \frac{\text{Total no. of pollens in 50 droplets}}{\text{Volume of 50 droplets}} \times \text{Vol. of ethanol}$$

Perianths of the flower were removed and the ovary was dissected carefully. A dissecting microscope was utilized to observe and calculate the amount of ovules developed in the ovary. Data for floral characteristics (number of flowers and bracteoles per inflorescence, and number of ovules) of *T. bibracteata* was also recorded in the greenhouse.

Presence of insect visitors and their behaviour and visit timing on stamens or stigmas were noted. These insect visitors were divided into two groups, which are visitors and pollinators, based on their behaviour and likelihood of aiding in pollination. According to Zhang et al. (2005), insects which were observed on inflorescence were considered as visitors whereas only those insects with deposition of pollen grains, and were found frequently interacting with anthers and stigmas were categorised as pollinators. Besides, some insects which acted as florivore were also recorded. This group of insects caused damages to inflorescences and flowers by consuming the floral structures and reproductive parts of an inflorescence.

Pollinator for the respective flower of species was determined by several aspects (frequency of visitation, the ability to contact floral reproductive organs, amount of pollen deposition on insect body, e.t.c; Chen et al., 2015; Petanidou & Potts, 2006; Bosch et al., 2009). During the observations, all the insect visitors were further grouped into midge, mosquito, stingless bee, ant and other insects which weren't detected as frequent as the earlier four groups. These other insects included spider, beetle, fruit fly, and grasshopper.

The number of flowers which were visited by certain types of insect group were recorded to calculate the rate of insect visitation on flowers. Each observation was conducted for three to five hours. A visit was defined as any contact with the floral parts of the inflorescence. The rate of insect visitation on flowers was calculated by dividing the number of flowers visited by a group of insect with the total of flowers observed.

$$\text{Rate of insect visitation on flowers} = \frac{\text{Number of flowers visited by the insect group}}{\text{Total of observed flowers}}$$

The floral parts (anthetic flower, post-anthetic flower, bract and bracteole) landed by the insect group were also noted. Since it was very difficult to capture all insects due to their short stay on the inflorescence, the highest number of insects for each insect group per timing were estimated during the watches. Photographs of insect visitors were taken for identification purpose. Observations under a dissecting microscope were necessary for tiny insects to observe detailed features for identification. Ethanol-preserved insect specimens of midges were sent to Dr Art Borkent (Royal British Columbia Museum and the American Museum of Natural History) for further identification.

4.2.4 Field manipulation experiment

Field manipulation experiment was conducted to experiment on the purpose of long filiform bracteoles and showy bracts development of *T. borneensis*, *T. cristata*, *T. laevis* and *T. reducta* (Zhang et al., 2005). This experiment tested on the effect on pollination and fruit development by the absence of those floral accessories. In this experiment, the tested subjects must be inflorescence buds which were tagged one week before their anthesis. There were a total of two treatments in this experiment. In each treatment, individuals were selected

by choosing an inflorescence bud randomly in the field. The number of inflorescence replicates in treatment was indicated in Table 4.5. The settings of these selected individuals were located at their natural habitat where natural pollination occurs. The two treatments were: (1) a non-manipulated control (seed set result from this treatment was also used in controlled pollination experiment) and (2) the removal of bracts and bracteoles. In the second treatment, no damage was incurred on the flower buds as injuries on flowers may affect the pollination and fruit development. Approximately two to four months later when fruits mature, the fruit set (fruit: flower ratio) and seed set (seed: ovule ratio) of inflorescence were counted and calculated.

4.2.5 Controlled pollination experiment

Controlled pollination experiment was designed to determine whether *T. borneensis*, *T. laevis* and *T. reducta* were self or cross or mixed pollinated plant. This experiment also targeted on examining the pollination function of insect visitors toward *Tacca* spp. (Zhang et al., 2005). Three pollination treatments were conducted on *Tacca* spp. at their natural habitat. Treatment 1 was open-pollinated control which was the same as in control for field manipulation experiment; treatment 2 was artificial self-pollination; treatment 3 was artificial cross-pollination. For treatment 2 and 3, the number of inflorescence replicates was indicated in Table 4.5. In treatment 2, all the selected inflorescences were bagged before their bracts open to limit the accessibility of insect visitors toward the flowers. On the day of anthesis, the flowers were emasculated and their own pollen was artificially deposited on the stigma. In treatment 3, in addition to bagging the selected inflorescence, the flowers on inflorescences were emasculated one or two days before anthesis to prevent self-pollination.

Hand cross-pollinations were performed. Pollen was sourced from plants up to 5 m away from the experimented plants. Samples from both treatments were bagged throughout the whole experiment to prevent any insects from approaching the flowers. After two to four months, fruits produced were collected. Seed sets of each treatment were recorded and analysed. The number of inflorescence replicates was indicated in Table 4.5. In addition, autonomous tests were performed to test the capability of autonomous self-pollination among *Tacca* spp. in west Sarawak. In this test, the inflorescence bud was bagged without performing emasculation on flowers. The ability of *Tacca* inflorescence to produce fruits without pollinator was assessed.

Table 4.5: Number of inflorescence replicates for each species on field manipulation experiment, controlled pollination experiment and autonomous pollination test.

Species	Field manipulation experiment	Controlled pollination experiment		Autonomous pollination test
		Self	Cross	
<i>T. borneensis</i>	4	2	2	2
<i>T. laevis</i>	7	9	7	2
<i>T. reducta</i>	28	16	24	5
<i>T. cristata</i>	13	–		1

4.2.6 Data analysis

For each species, mean and standard error, including the range value of the number of flowers per inflorescence, number of bracteoles per inflorescence, number of fruits per infructescence, fruit set, number of seeds per fruit and seed set were calculated. The coefficient of variation (CV) of the number of flowers, bracteoles, fruits, seeds, and ovules of each species was calculated. The equation of CV is as follows.

$$\text{Coefficient of Variance} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

In each experiment, fruits developed were collected for each sample. The number of fruits and their respective seed number were also recorded. Data from pollen and ovules count were used to calculate pollen: ovule (P:O) ratio of *Tacca* species. P:O ratio predicts whether the flower is performing self-fertilization or cross-fertilization (obligate autogamy = 27.7, facultative autogamy = 168.5; Cruden, 1977). Low P:O ratio correlates with self-fertilizing plants. The formula of P:O ratio is shown below.

$$\text{Pollen : ovule ratio} = \frac{\text{Number of pollen grain per flower}}{\text{Number of ovules per flower}}$$

Software Past 3.0 (Hammer et al., 2001) was used to analyse all the data. The mean and standard error of each collected data were calculated using the software. Intraspecific data on fruit and seed set from field manipulation experiment were analysed using Welch's t-test. In controlled pollination experiment, only seed set data per species for all three treatments were assessed by Welch's one-way ANOVA. Lastly, Mann-Whitney pairwise post-hoc test with Bonferroni corrected p values was used for results which shown significant difference in ANOVA.

4.3 Results

4.3.1 Flowering period

After one year of observation, the flowering periods of all six species were recorded monthly, and the flowering period patterns varied among *Tacca* species (Table 4.6). *Tacca laevis* and *T. reducta* bloomed throughout the year. From the year 2017 to 2018, *T. laevis* had 66.7% of inflorescences (N = 24) developed into infructescences, whereas *T. reducta* had 71.1% of inflorescences (N = 45) developed into infructescences.

Tacca cristata from FRIM was recorded to be developing inflorescences every month. Fifty per cent of *T. cristata*'s inflorescences (N = 30) developed into infructescences. *Tacca borneensis* was also recorded to develop inflorescence all year long except the months of February and September. For *T. borneensis*, 61.5% of sampled inflorescences (N = 26) yielded fruits. Despite the small population size of *T. palmata*, the flowering events were recorded from December to January, March to April, and August to October. All sampled inflorescences of *T. palmata* (N = 4) produced fruits. For *T. leontopetaloides*, an inflorescence was found flowering in early September 2017. During the time, c. 15 infructescences were observed in the population, and thus there was flowering event during August or earlier. This species' rhizome remained dormant since January. In late July 2018, this species was found with infructescences (c. N = 20) again. The infructescences were probably produced from inflorescences bloomed in June or early July.

Although the inflorescences of *T. laevis* and *T. reducta* were found throughout the year, the number of inflorescences recorded every month varied (Table 4.7). For *T. laevis*, the months with the highest number of inflorescences recorded were March and November with 13 and 8 inflorescences, respectively at KS. During other months of the year, only one to four

inflorescences recorded. During May, no fieldwork was conducted in KS, but there were new inflorescence buds observed in late April. At KNP, flowering only occurred in April, July and October due to low population count in this area. For *T. reducta* at RRP, May and October had the most number of inflorescences with 15 and 38 inflorescences, respectively. *Tacca reducta* had at least five inflorescences for each month.

Flowering periods of *T. borneensis* in different locations were slightly different in which at MS, inflorescences were seen in March to May, August and November, at KNP inflorescences were seen in January, March, April, June, October and December, whereas at RRP only July and October (Table 4.6). *Tacca borneensis* had a rather low number of inflorescence (1 – 6) throughout the flowering period. MS had six inflorescences in April which was the highest number of inflorescences recorded for this species.

For *T. cristata*, 17 inflorescences were recorded in February. The rest of the observed period (April, July, August, October and November) had seven to ten inflorescences. Flowering of *T. palmata* was solitary, only one inflorescence was found in a locality whenever this species flowered. The highest number of inflorescences for *T. leontopetaloides* was not available due to insufficient data. The only recorded inflorescence was in September at PS.

Table 4.6: Flowering period and fruiting period of observed *Tacca* spp. throughout the years 2017 and 2018 (“X” represents the presence of inflorescence, “O” represents the presence of infructescence, “–” represents absent).

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<i>T. borneensis</i>	X –	– –	XO	XO	XO	X –	X –	X –	– O	XO	XO	XO
<i>T. laevis</i>	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO
<i>T. reducta</i>	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO
<i>T. palmata</i>	XO	– O	X –	XO	– O	– –	– –	X –	XO	XO	– O	XO
<i>T. leontopetaloides</i>	– –	– –	– –	– –	– –	X –	– O	XO	XO	– O	– O	– O
<i>T. cristata</i>	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO	XO

Table 4.7: Number of inflorescences recorded for each species monthly from the year 2017 to 2018. Locality codes were added in parentheses behind the number of inflorescences in that population (“–” represents no field observation was conducted during the month).

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<i>T. borneensis</i>	3 (KNP)	–	1 (MS), 2 (KNP)	6 (MS), 1 (KNP)	2 (MS)	1 (KNP)	1 (RRP)	2 (MS)	0	2 (KNP), 1 (RRP)	4 (MS)	2 (KNP)
<i>T. laevis</i>	1 (KS)	4 (KS)	13 (KS)	4 (KS), 2 (KNP)	–	1 (KS)	3 (KS), 1 (KNP)	1 (KS)	1 (KS)	1 (KNP)	8 (KS)	3 (KS)
<i>T. leontopetaloides</i> (PS)	0	–	0	0	0	–	0	–	1	0	0	0
<i>T. palmata</i>	1 (PS), 1 (RRP)	0	1 (RRP)	1 (KPL)	0	0	0	1 (PS)	1 (PS)	1 (PS), 1 (RRP)	0	1 (RRP)
<i>T. reducta</i> (RRP)	7	5	11	11	15	5	10	10	10	38	11	7
<i>T. cristata</i>	–	17	–	8	–	–	7	8	–	10	7	–

4.3.2 Flowering phenology

4.3.2.1 *Tacca borneensis*

The number of flower blooming per inflorescence per day of *T. borneensis*' inflorescences varied from one to fourteen flowers. If there were more than one flower bloomed per inflorescence, the flowers started anthesis either at the same time or about 10 to 15 minutes delayed between flowers. The sequence of blooming flowers began from the middle or most inside buds, then the adjacent flower(s) bloomed on the next day, and lastly, outer most bud(s) bloomed on the last day. The blooming duration of an inflorescence depended on the number of flowers developed. While an inflorescence normally has 10 or more flowers, the usual anthesis period was relatively short with only four to five consecutive days, and some can even last for a week depending on the number of flowers of the inflorescence. Before anthesis began, outer involucral bracts of *T. borneensis* inflorescence surrounded inner bracts, bracteoles and flower buds. When the inflorescence bloomed, the outer pair of bracts reflexed, and the inner pair of bracts positioned upright with middle flower buds erected and adjacent flower buds either erected or horizontal orientated. Middle flower buds bloomed on the subsequent day, and the adjacent young flower buds, which will bloom on the next day, was erected. The flowers of this species began anthesis from about 07:00 to 09:00 (local time, UTC + 0800; N = 6), and lasted for 8 to 10 hours. Outer perianths reflexed from 07:39 to 08:50, and then inner perianths reflexed from 08:27 to 09:54.

The stigma was observed to have sticky droplets one day before the flower bloomed. Whereas, the anthers were only found to dehisce pollen about one hour after the flower opened (10:10 to 11:00). The blooming flowers remained stiffly upright during the anthetic period and then became decumbent at 15:00 to 17:00 of the same day. No other floral

movement was observed. Filiform bracteoles only shook with the wind. No detectable odour was present in the flowers of *T. borneensis*.

After all the flowers finished blooming, the base of peduncle began to twist in two days, and the inflorescence finally declinated on the ground. Fertilized flowers were developed into purplish dark green, obpyramidal-shaped fruits with six rib edges. The perianths sometimes persisted. After about three months, the fruit ripens. Brownish black, harden seeds were developed within the matured fruit. A single plant of *T. borneensis* sometimes developed up to four sets of inflorescence at different phases (inflorescence bud and flowering inflorescence) at the same time. Commonly, a *T. borneensis* plant goes into a resting period of more than two months after the previous inflorescence completed anthesis. Some *T. borneensis* plants were never seen flowering or fruiting for more than six months. Figure 4.10 showed the stages of anthesis for *T. borneensis*. Figure 4.11 showed the cross-section of a flower in anthesis, anthers before and after anthesis, and stigma of flower bud, anthetic flower and post-anthetic flower under dissecting microscope.



Figure 4.10: *Tacca borneensis*. **A - B:** Inflorescence bud. **C:** Inflorescence with fully expanded bracts. **D:** Flower buds. **E:** Perianths reflexed. **F:** Anthetic flowers. **G:** Post-anthetic flowers.

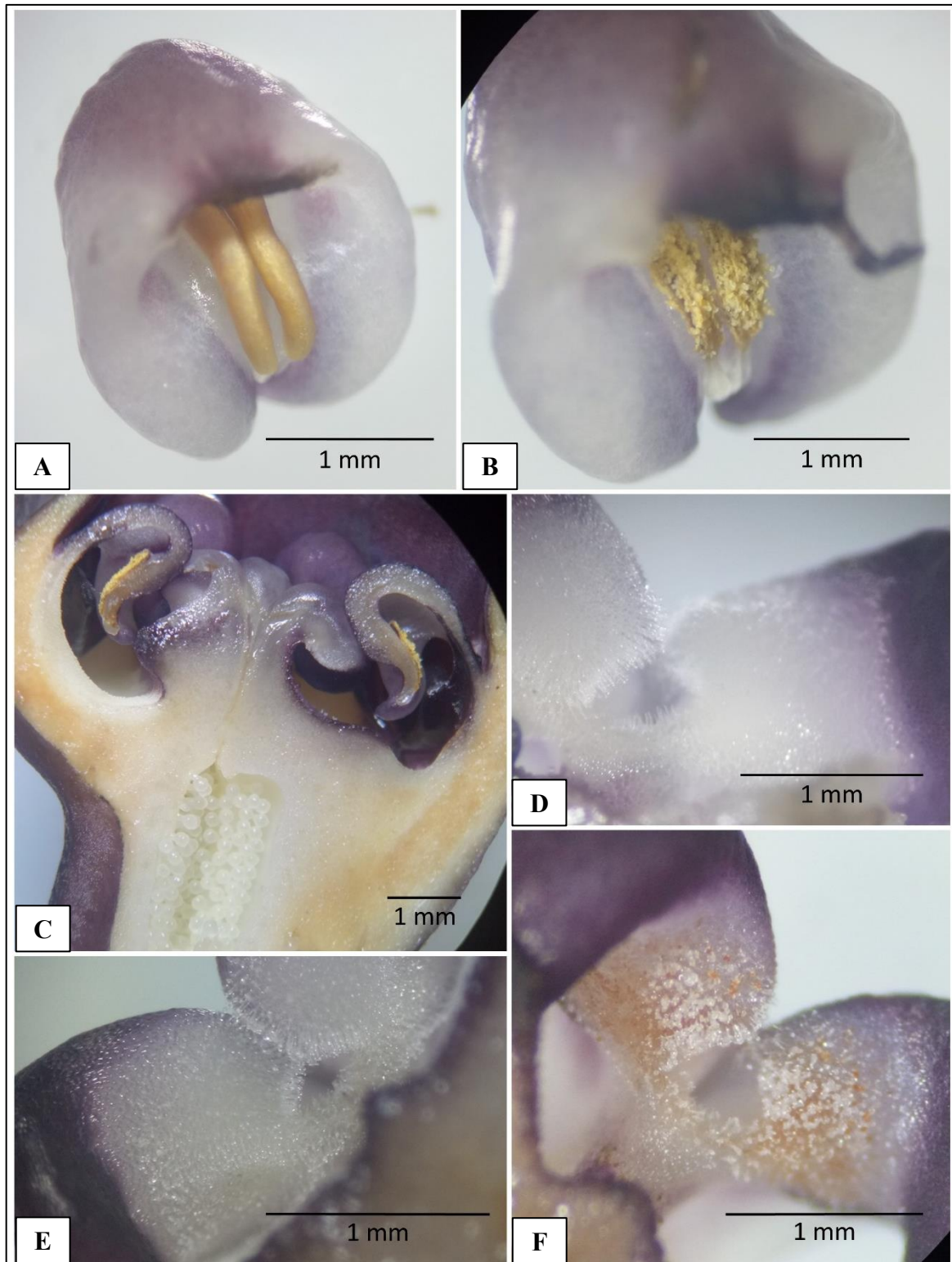


Figure 4.11: Anthers and stigma of *T. borneensis*. **A:** Anther of flower bud. **B:** Anther of anthetic flower. **C:** Cross-section of anthetic flower. **D:** Stigma of flower bud. **E:** Stigma of anthetic flower. **F:** Stigma of post-anthetic flower.

4.3.2.2 *Tacca laevis*

Inflorescence of *T. laevis* blooms one or two flowers per day in general. If there were two flowers blooming per inflorescence, the flowers started anthesis at the same time or about 10 to 15 minutes delayed between flowers. The sequence of blooming flowers began from the middle or most inside bud, then the adjacent flower(s) bloomed on the next day, and lastly, outer most bud(s) bloomed on the last day. The blooming duration of the inflorescence is dependent on the number of flowers developed. Most of the inflorescences have anthetic period about three to five days, and some can even last for a week depending on the number of flowers of the inflorescence. It is also usual to observe no flower blooming in a day throughout an inflorescence anthesis period. Before anthesis begins, outer involucral bracts of *T. laevis* inflorescence surrounded inner bracts, bracteoles and flower buds. When the inflorescence bloomed, the outer pair of bracts reflexed, and the inner pair of bracts positioned upright with middle flower buds horizontally orientated and adjacent flower buds declinate. Middle flower buds erected and bloomed on the subsequent day, and the adjacent young flower buds, which will bloom on the next day, became horizontally oriented. The flowers of this species began anthesis from about 05:00 to 06:00 (UTC + 0800; N = 5), and lasted for 13 to 14 hours. Outer perianths reflexed from 05:41 to 05:53, and then inner perianths reflexed from 05:50 to 06:00.

The stigma was observed to have sticky droplets one day before the flower will bloom. Whereas, the anthers were only found to dehisce pollen between three to four hours after the flower opened (09:00 to 10:00). The blooming flowers remained stiffly upright during the anthetic period and then became decumbent at 18:00 to 19:00 of the same day. No other floral movement was observed. Filiform bracteoles only shook with the wind. A faint odour was emitted by flowers throughout the blooming period.

After all the flowers finished blooming, the base of peduncle began to twist in two days, and the inflorescence finally declinated on the ground. Fertilized flowers were developed into glossy green, obpyramidal-shaped fruits with six rib edges. The perianths sometimes persisted. After about three months, the fruit ripens. Light brownish, harden seeds were developed within the matured fruit. A single plant of *T. laevis* sometimes developed up to three sets of inflorescence at different phases (inflorescence bud and flowering inflorescence) at the same time. Commonly, a *T. laevis* plant goes into a resting period of more than two months after the previous inflorescence completed anthesis. Figure 4.12 showed the stages of anthesis for *T. laevis*. Figure 4.13 showed anther and stigma of *T. laevis* during anthesis under a dissecting microscope.

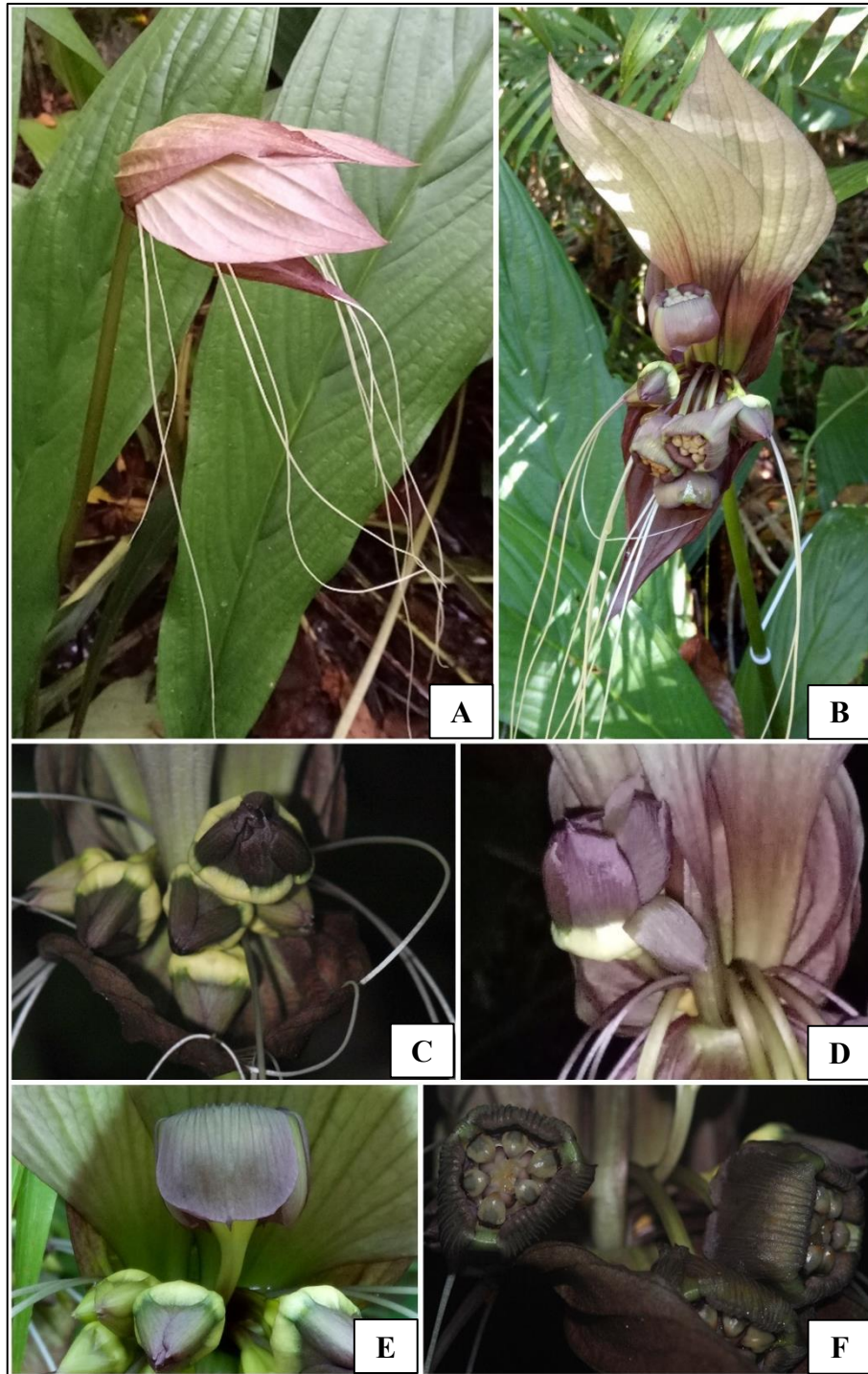


Figure 4.12: *Tacca laevis*. **A:** Inflorescence bud. **B:** Inflorescence with fully expanded bracts. **C:** Flower buds. **D:** Perianths reflexed. **E:** Anthetic flowers. **F:** Post-anthetic flowers.

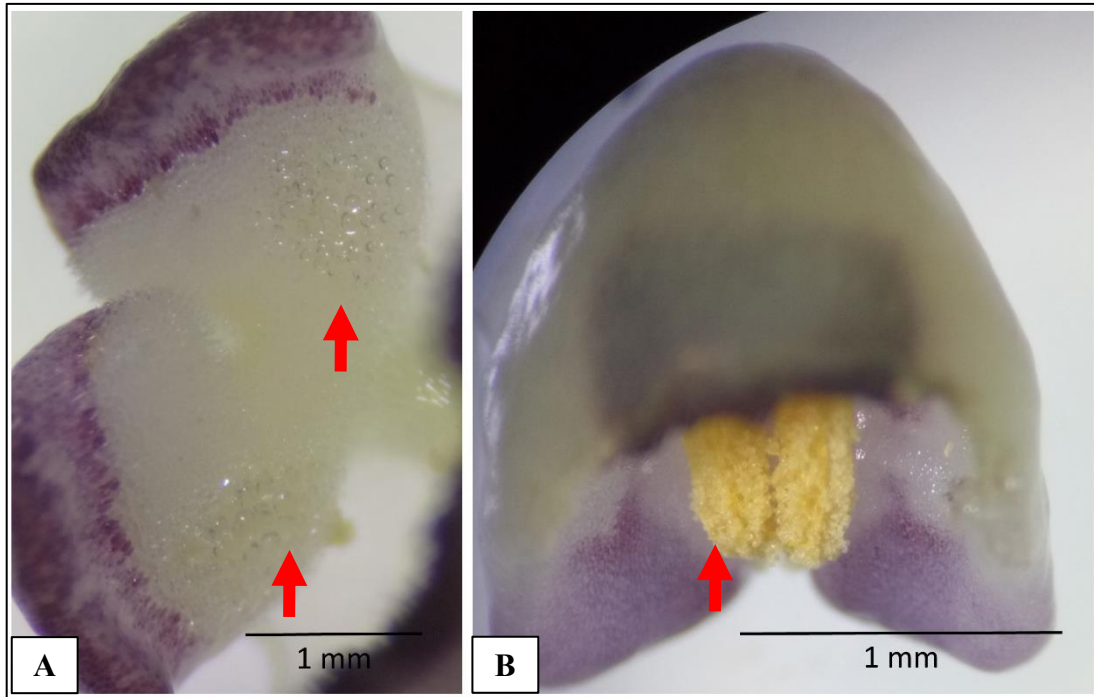


Figure 4.13: Anther and stigma of *T. laevis*. **A:** Stigma of anthetic flower (arrows). **B:** Anther of anthetic flower (arrow).

4.3.2.3 *Tacca leontopetaloides*

Inflorescence of *T. leontopetaloides* blooms two to eleven flowers per day. If there were more than one flower blooming per inflorescence, the flowers started anthesis at the same time or about 10 to 15 minutes delayed between flowers. Sequence of blooming flowers began from the middle or most inside buds, then the adjacent flower(s) bloomed on the next day, and lastly outer most bud(s) bloomed on the last day. An inflorescence with 22 flowers had anthesis period lasted for five days. The flowers of this species began anthesis from about 17:40 to 19:00 (UTC + 0800; N = 1), and lasted for 11 to 12 hours. Outer perianths reflexed from 17:41 to 18:00, and then inner perianths reflexed from 18:16 to 19:10.

The stigma were observed to have sticky droplets one day before the flower will bloom. The blooming flowers remained stiffly upright during the anthetic period. The perianths only slightly opened during anthesis. During post anthesis, the perianths of the flowers were

closed, covering the reproductive parts, and then the flowers became decumbent at 05:00 to 06:00 of the next day. No other floral movement was observed. Filiform bracteoles only shook with the wind. A faint odour was emitted by flowers throughout the blooming.

After all the flowers finished blooming, the peduncle remained standing until the fruits developed. Fertilized flowers were developed into green, round fruits with six ribs. The perianths persisted. After about two months, the fruit ripen. Light brown, irregular, harden seeds with white testa were developed within the matured fruit. Figure 4.14 showed the stages of anthesis for *T. leontopetaloides*.

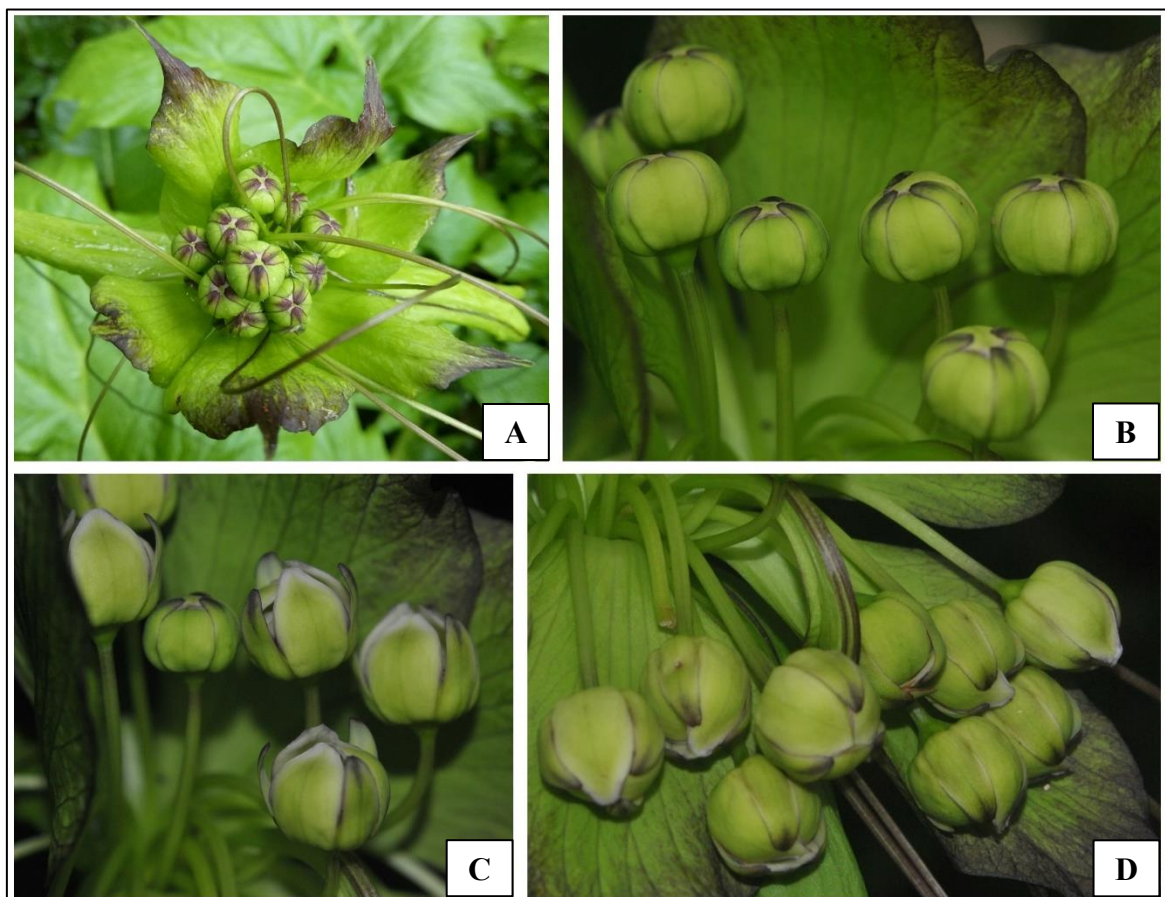


Figure 4.14: *Tacca leontopetaloides*. **A:** Inflorescence with fully expanded bracts (Plan view) **B:** Flower buds. **C:** Anthetic flowers. **D:** Post-anthetic flowers.

4.3.2.4 *Tacca palmata*

Inflorescence of *T. palmata* blooms one to three flowers per day in general. If there were more than one flower blooming per inflorescence, the flowers started anthesis at the same time or about 10 to 15 minutes delayed between flowers. Sequence of blooming flowers began from the middle or most inside buds, then the adjacent flower(s) bloomed on the next day, and lastly outer most bud(s) bloomed on the last day. The blooming duration of the inflorescence is dependent on the number of flowers developed. Most of the inflorescences have anthetic period six days to two weeks. Before anthesis begins, outer involucral bracts of *T. palmata*'s inflorescence separated laterally, and inner involucral bracts in the middle with an upright position. The undeveloped flower buds, in an erected state, located between the bract pairs. The flowers of this species began anthesis from about 06:00 to 08:00 (UTC + 0800; N = 4), and lasted for 12 to 13 hours. Both outer and inner perianths reflexed from 06:40 to 07:44.

The stigma were observed to have sticky droplets one day before the flower will bloom. The blooming flowers remained stiffly upright during the anthetic period. During post anthesis, the perianths of the anthetic flowers were closed, covering the reproductive parts. The flowers sometimes became decumbent at 18:00 to 19:00 of the same day. Some flowers remained erect until the fruit developed if fertilization occurred. No other floral movement was observed. No detectable odour was present in the flowers of *T. palmata*.

After all the flowers finished blooming, the peduncle remained standing until the fruits developed. Fertilized flowers were developed into green, round fruits. The perianths persisted. After about three months, the fruit turned yellow and then red when ripen. Brown,

irregular, harden seeds were developed within the matured fruit. Figure 4.15 showed the stages of anthesis for *T. palmata*.

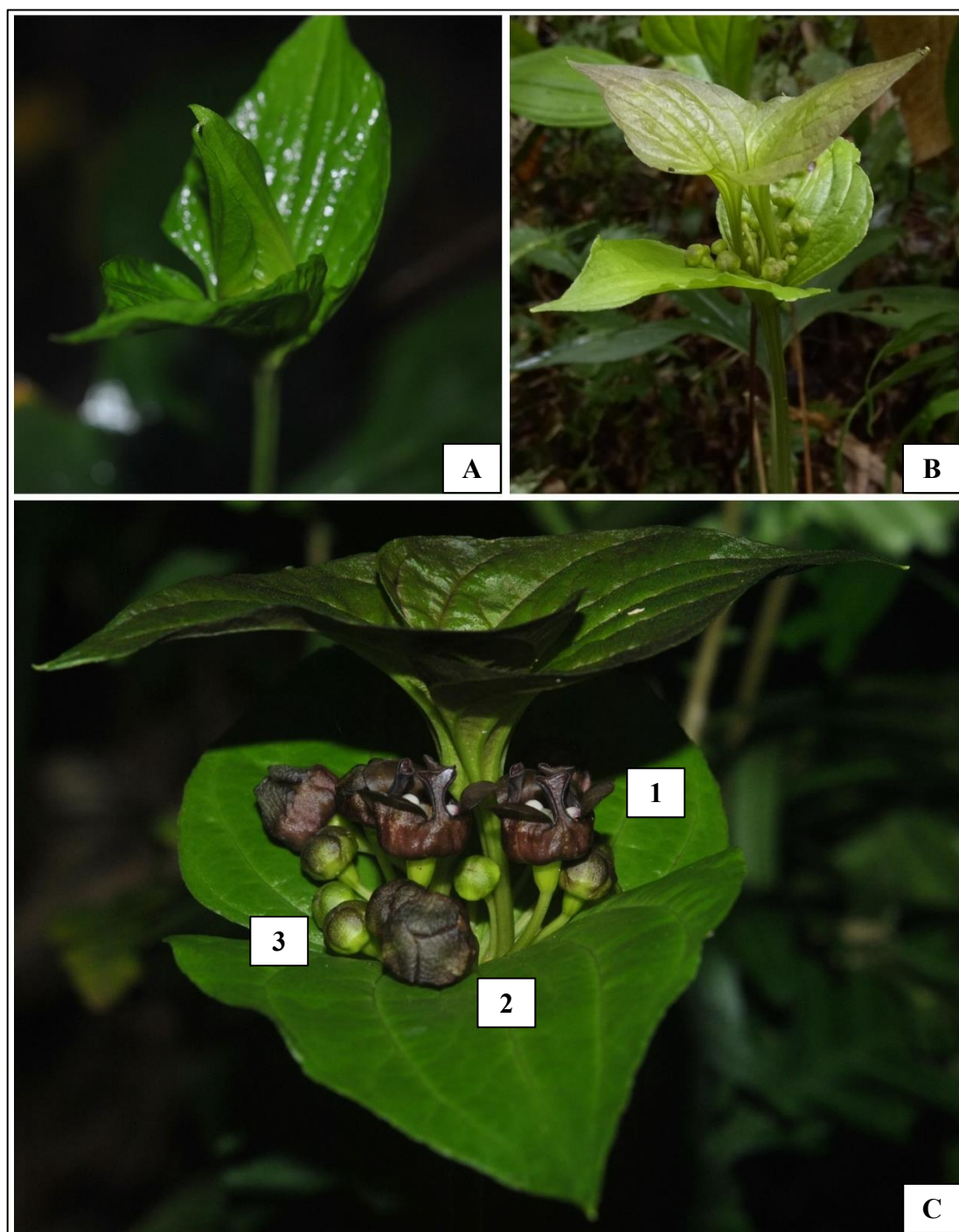


Figure 4.15: *Tacca palmata*. **A:** Inflorescence bud. **B:** Inflorescence with fully expanded bracts. **C (1 – 3):** Inflorescence (1: Anthetic flower; 2: Post-anthetic flower; 3: Flower bud).

4.3.2.5 *Tacca reducta*

Generally, an inflorescence of *T. reducta* blooms one or two flowers per day. If there were two flowers blooming per inflorescence, the flowers started anthesis at the same time or about 10 to 15 minutes delayed between flowers. Sequence of blooming flowers began from the middle or most inside bud, then the adjacent flower(s) bloomed on the next day, and lastly outer most bud(s) bloomed on the last day. The blooming duration of inflorescence is dependent on the number of flowers developed. Thus, each inflorescence lasted for a different number of days, varying from two days to a week. Most of the inflorescences bloomed for a consecutive three or four days. More often, an inflorescence has a day pause during the anthetic period in which no flower blooming on that day. Before anthesis began, four similar sizes, linear-triangular bracts covered all the flower buds. When the inflorescence bloomed, the bracts reflexed, and the middle-most flower buds were in an erect position whereas other flower buds were in a horizontal position. Middle flower buds bloomed on the subsequent day, and the adjacent flower buds, which will bloom on the next day, erected. The flowers of this species began anthesis from about 07:00 to 08:00 (UTC + 0800; N = 8), and lasted for 11 to 12 hours. Outer perianths reflexed from 07:33 to 08:10, and then inner perianths reflexed from 08:20 to 09:02.

The stigma were observed to have sticky droplets one day before the flower will bloom. Whereas, the anthers were only found to dehisce pollen between two to three hours after the flower opened (10:00 to 11:00). The blooming flowers remained stiffly upright during the anthetic period and then became decumbent at 18:00 to 19:00 of the same day. No other floral movement was observed. Filiform bracteoles only shook with the wind. No detectable odour was present in the flowers of *T. reducta*.

After all the flowers finished blooming, the base of peduncle began to twist in two days, and the inflorescence finally declinated on the ground. Fertilized flowers were developed into dark purplish, obpyramidal-shaped fruits with six rib edges. The perianths sometimes persisted. After about three months, the fruit ripens. Brownish black, harden seeds were developed within the matured fruit. A single plant of *T. reducta* sometimes developed two sets of inflorescence at different phases (inflorescence bud and flowering inflorescence) at the same time. Commonly, a *T. reducta* plant goes into a resting period of one to three months after the previous inflorescence completed anthesis. Figure 4.16 showed the stages of anthesis for *T. reducta*. Figure 4.17 showed the cross-section of bud and anthetic flower, anthers of anthetic flower, and stigma of anthetic and post-anthetic flower.



Figure 4.16: *Tacca reducta*. **A:** Inflorescence bud. **B:** Inflorescence with fully expanded bracts. **C:** Perianths reflexed. **D:** Anthetic flowers. **E:** Post-anthetic flowers.

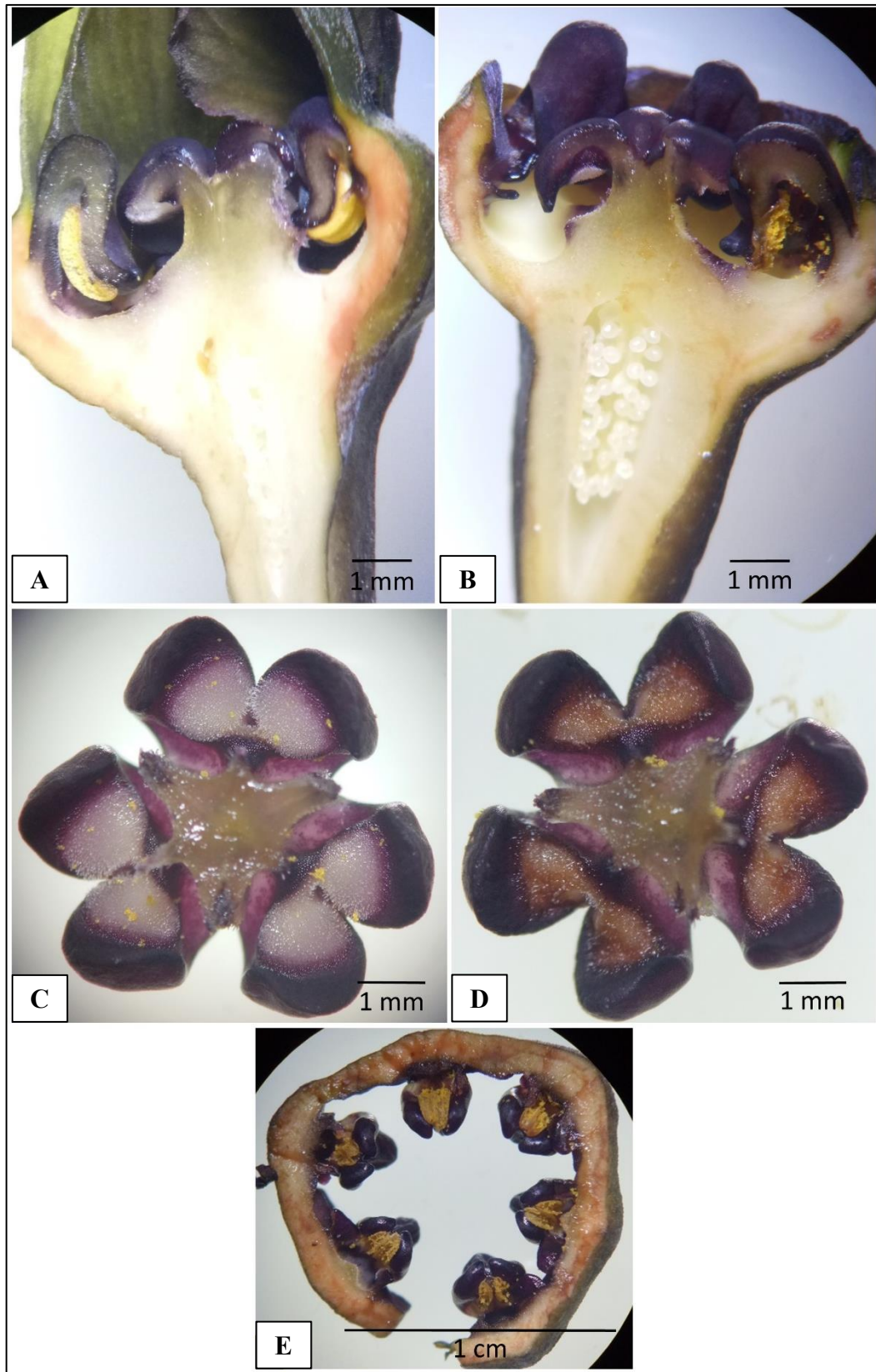


Figure 4.17: Anthers and stigma of *T. reducta*. **A:** Cross-section of flower bud. **B:** Cross-section of anthetic flower. **C:** Stigma of anthetic flower. **D:** Stigma of post-anthetic flower. **E:** Anthers of anthetic flower.

4.3.2.6 *Tacca cristata*

Inflorescence of *T. cristata* blooms one to three flowers per day in general. If there were more than one flower blooming per inflorescence, the flowers started anthesis at the same time or about 10 to 15 minutes delayed between flowers. The sequence of blooming flowers began from the middle or most inside buds, then the adjacent flower(s) bloomed on the next day, and lastly, outer most bud(s) bloomed on the last day. The blooming duration of an inflorescence is dependent on the number of flowers developed. Most of the inflorescences have anthetic period four days to a week, and some can even last for up to 10 days depending on the number of flowers of the inflorescence. It is also usual to observe no flower blooming in a day throughout anthetic period of an inflorescence. Before anthesis begins, outer involucral bracts of *T. cristata* inflorescence surrounded inner bracts, bracteoles and flower buds. When the inflorescence bloomed, the outer pair of bracts separated, and the inner pair of bracts positioned upright with all flower buds either horizontal orientated or declined. Middle flower buds erected and bloomed on the subsequent day, and the adjacent young flower buds, which will bloom on the next day, remained horizontal or pendent. The flowers of this species began anthesis from about 06:00 to 07:00 (UTC + 0800; N = 5), and lasted for 12 to 13 hours. Outer perianths reflexed from 05:48 to 06:34, and then inner perianths reflexed from 06:38 to 07:00.

The stigma was observed to have sticky droplets one day before the flower will bloom. The blooming flowers remained stiffly upright during the anthetic period and then became decumbent at 18:00 to 19:00 of the same day. No other floral movement was observed. Filiform bracteoles only shook with the wind. No detectable odour was present in the flowers of *T. cristata*.

After all the flowers finished blooming, the base of peduncle began to twist in two days, and the inflorescence finally declinated on the ground. Fertilized flowers were developed into dark purple, obpyramidal-shaped fruits with six rib edges. The perianths sometimes persisted. After about three months, the fruit ripens. Dark brown, harden seeds were developed within the matured fruit. A single plant of *T. cristata* sometimes developed up to two sets of inflorescence at different phases (inflorescence bud and flowering inflorescence) at the same time. Commonly, a *T. cristata* plant goes into a resting period of more than two months after the previous inflorescence completed anthesis. Figure 4.18 showed the stages of anthesis for *T. cristata*.

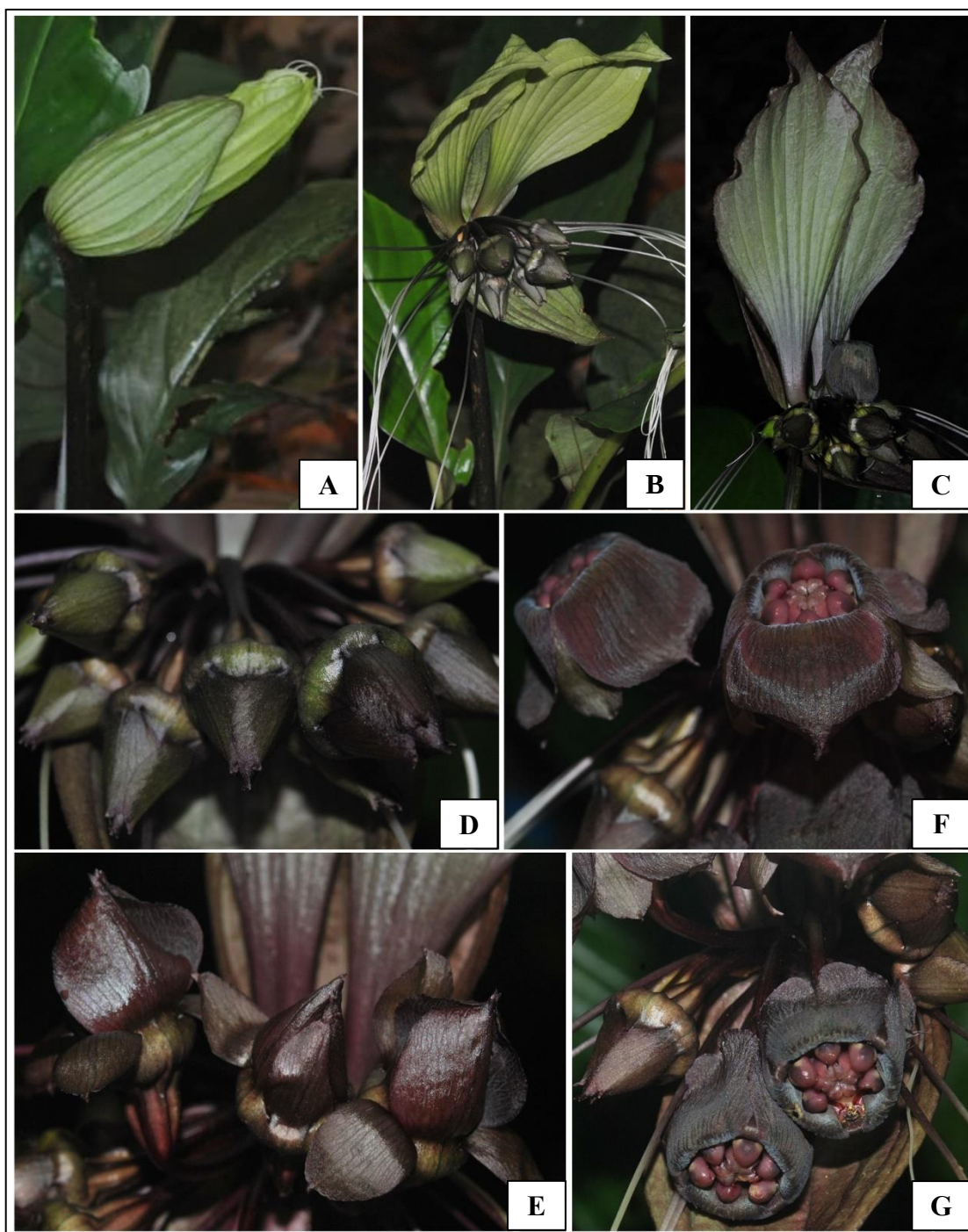


Figure 4.18: *Tacca cristata*. **A - B:** Inflorescence bud. **C:** Inflorescence with fully expanded bracts. **D:** Flower buds. **E:** Perianths reflexed. **F:** Anthetic flowers. **G:** Post-anthetic flowers.

Summary of floral phenology for all species was recorded in Table 4.8.

Table 4.8: The time period of each floral movements (including perianths blooming, pollen extrusion, and flower declinate) for each species. The presence of floral scent was also recorded.

Species	No. of inflorescences	Time period of floral movements					Presence of floral scent	Infructescence position
		Outer perianths opening	Inner perianths opening	Pollen extrusion	Flower declinate	Anthesis duration (hours)		
<i>Tacca borneensis</i>	6	07:39 – 08:50	08:27 – 09:54	10:10 – 11:00	15:50 – 17:18	8 – 10	No	Declinate
<i>Tacca laevis</i>	5	05:41 – 05:53	05:50 – 06:00	09:00 – 10:00	18:36 – 19:00	13 – 14	Faint, throughout anthetic period	Declinate
<i>Tacca leontopetaloides</i>	1	17:41 – 18:00	18:16 – 19:10	–	05:00 – 05:40	11 – 12	Faint, throughout anthetic period	Upright
<i>Tacca palmata</i>	4	06:40 – 07:44		–	18:50 – 19:30	12 – 13	No	Upright
<i>Tacca reducta</i>	8	07:33 – 08:10	08:20 – 09:02	10:00 – 11:00	17:42 – 18:13	10 – 11	No	Declinate
<i>Tacca cristata</i>	5	05:48 – 06:34	06:38 – 07:00	–	18:19 – 18:50	12 – 13	No	Declinate

4.3.3 Floral Characteristics

4.3.3.1 *Tacca bibracteata*

Tacca bibracteata generally produced three to four flowers per inflorescence. The mean for the number of flowers per inflorescence was 2.8 ± 0.4 (N = 5). The number of hair-like bracteoles per inflorescence ranged from 14 to 16 with an average value of 14.6 ± 0.4 (N = 5). Since this species was only observed within greenhouse, no fruit was produced, and thus no seed set too. The number of pollen was not estimated. Besides, the number of ovules ranged from 42 to 49, with mean value equal to 45.3 ± 0.8 (N = 9). Table 4.9 summarized the statistical floral characteristics.

4.3.3.2 *Tacca borneensis*

Average flower number of *T. borneensis* produced by an inflorescence is 17 ± 0.9 (N = 39; Table 4.9). The minimum number of flowers recorded was 7 flowers whereas the maximum number was 25 flowers. However, the number of bracteoles for this species was lower than the other species in general. It had only 3 to 10 bracteoles recorded per inflorescence with mean of 5.8 ± 0.2 (N = 38). Although *T. borneensis* produced inflorescence with more flowers, the fruit set percentage for the sampled individual was very low with mean value of $19.5\% \pm 2.8$ (N = 16). The range of fruit set percentage was from 4.2% to 54.5%. The number of fruits produced for sampled inflorescence was one to six fruits. Whereas, in the field, the highest number of fruits recorded is 11 per infructescence. The seed number developed in each fruit varied from 9 to as much as 305. Hence, the seed set percentage was ranged from 3.8% to 128% with mean value of $55.1\% \pm 2.8$ (N = 117). The estimated number of pollen grains ranged from 6770 to 15530, with mean value equal to 10041.3 ± 891.6 (N = 8). The

number of ovules for *T. borneensis* is highest among other examined species with average 238.3 ± 11.2 (N = 16). The ovules number per flower varied from 131 to 308. As a result, the P:O ratio of *T. borneensis* is 42.1.

4.3.3.3 *Tacca laevis*

Tacca laevis was recorded to have a minimum of two flowers to a maximum of 12 flowers on an inflorescence. Average flowers number of this species is 6.1 ± 0.3 (N = 62; Table 4.9). The number of bracteoles per inflorescence of *T. laevis* ranged from 6 to 13, and the mean value is 8.9 ± 0.2 (N = 61). The number of fruits per infructescence ranged from one to 12 fruits. Most of the sampled inflorescence had all flowers developed into fruits. Thus, the fruits set for this species was considerably high which is $81.6\% \pm 6.8$ (N = 17). Seed set percentage had a mean value equal to 37.3 ± 1.6 (N = 135) with maximum percentage of 94.4%, and minimum percentage of 3.9%. The actual seed number varied from 6 to 144 seeds per fruit. The estimated number of pollen grains ranged from 8240 to 13170, with mean value equal to 11331.1 ± 580.7 (N=8). The average ovule number was 152.6 ± 2.2 (N = 18). The range of ovule number was from 138 to 168 ovules per flower. As a result, the P:O ratio of *T. laevis* is 74.3.

4.3.3.4 *Tacca leontopetaloides*

Tacca leontopetaloides was recorded to produce seven to 145 flowers per inflorescence. The mean for the number of flowers per inflorescence was 46.8 ± 15 (N = 9; Table 4.9). Number of hair-like bracteoles per inflorescence ranged from 9 to 57 with average value 22.4 ± 5 (N

= 9). Percentage of sampled fruit set per inflorescence was rather varying (13.6% – 78.6%) with mean value $40.5\% \pm 7.2$ (N = 9). The percentage value of seed set per fruit was moderate for this species. All sampled fruits have seed number ranging from as low as 9 to 73 seeds. Hence, resulted in the overall mean seed set percentage was 70.2 ± 1.7 (N = 75). Number of pollen was not estimated. Besides, the number of ovules ranged from 64 to 76, with mean value equal to 70.6 ± 0.9 (N=14).

4.3.3.5 *Tacca palmata*

Tacca palmata was recorded to produce 13 to 23 flowers per inflorescence (Table 4.9). The mean for the number of flowers per inflorescence was 17.6 ± 1.3 (N = 7). The percentage of sampled fruit set per inflorescence was rather varying (29.4% – 100%) with a mean value $54.5\% \pm 13.9$ (N = 5). The percentage value of seed set per fruit was moderate for this species. All sampled fruits have seed number ranging from as low as 1 to 18 seeds. Hence, resulted in the overall mean seed set percentage was $85.2\% \pm 3.1$ (N = 123). The number of pollen was not estimated. Due to limited inflorescence samples, only two flowers were dissected to count the ovules. Both of the flowers had eight ovules.

4.3.3.6 *Tacca reducta*

Tacca reducta generally produced two to five flowers per inflorescence (Table 4.9). The highest number of flowers per inflorescence recorded was up to nine flowers. The mean for the number of flowers per inflorescence was 4.7 ± 0.1 (N = 152). The number of hair-like bracteoles per inflorescence ranged from 10 to 18 with average value 14.3 ± 0.1 (N = 121). Percentage of sampled fruit set per inflorescence was rather varying (12.5% – 100%) with a

mean value $45.4\% \pm 4.3$ (N = 32). The percentage value of seed set per fruit was low for this species. All sampled fruits have seed number ranging from as low as 4 to 60 seeds. Hence, resulted in the overall seed set percentage lower than 50%, and the mean percentage value was only $27.7\% \pm 1.5$ (N = 85). The estimated number of pollen grains ranged from 7280 to 13140, with mean value equal to 10184.4 ± 654.0 (N=10). Besides, the number of ovules ranged from 99 to 128, with mean value equal to 109.4 ± 2.0 (N=16). As a result, the P:O ratio of *T. reducta* is 93.1.

4.3.3.7 *Tacca cristata*

Tacca cristata produced four to 19 flowers per inflorescence (Table 4.9). The mean for number of flowers per inflorescence was 9.9 ± 0.4 (N = 67). Number of bracteoles per inflorescence ranged from 10 to 25 with average value 14.9 ± 0.4 (N = 53). Percentage of sampled fruit set per inflorescence varied from 7.1% – 80% with mean value $35.3\% \pm 6.0$ (N = 15). All sampled fruits have seed number ranging from 8 to 156 seeds. The mean percentage value of seed set was only $42.2\% \pm 3.1$ (N = 48). The estimated number of pollen grains ranged from 7670 to 13870, with mean value equal to 10526 ± 1351.6 (N = 5). Besides, the number of ovules ranged from 155 to 235, with mean value equal to 195.8 ± 5.9 (N = 15). As a result, the P:O ratio of *T. cristata* is 53.8.

All the calculated P:O ratio of *Tacca* spp. fall between 27.7 (obligate autogamy) and 168.5 (facultative autogamy). The CV of flowers were generally less than 40 %, except *T. leontopetaloides* with CV equals to 96.5 %. In addition, the CV of *T. leontopetaloides* for bracteoles number was also the highest with value 67 %. Other species had CV less than 30 % for number of bracteoles. For thenumber of fruits, all species had CV more than 45 %.

Table 4.9: Overall floral characteristics for all examined *Tacca* spp. (“–” represented no data obtained).

Species	No. of flowers/ inflorescence	No. of bracteoles	Fruit set (%)	Seed set (%)	Pollen grains	Ovules	P:O ratio
<i>Tacca bibracteata</i>	2.8 ± 0.4 (2 – 4; N = 5)	14.6 ± 0.4 (14 – 16; N = 5)	–	–	–	45.3 ± 0.8 (42 – 49; N = 9)	–
<i>Tacca borneensis</i>	17 ± 0.9 (7 – 25; N = 39)	5.8 ± 0.2 (3 – 10; N = 38)	19.5 ± 2.8 (4.2 – 54.5; N = 16)	55.1 ± 2.8 (3.8 – 128; N = 117)	10041.3 ± 891.6 (6770 – 15530; N = 8)	238.3 ± 11.2 (131 – 308; N = 16)	42.1
<i>Tacca laevis</i>	6.1 ± 0.3 (2 – 12; N = 62)	8.9 ± 0.2 (6 – 13; N = 61)	81.6 ± 6.8 (12.5 – 100; N = 17)	37.3 ± 1.6 (3.9 – 94.4; N = 135)	11331.1 ± 580.7 (8240 – 13170; N = 8)	152.6 ± 2.2 (138 – 168; N = 18)	74.3
<i>Tacca leontopetaloides</i>	46.8 ± 15.0 (7 – 145; N = 9)	22.4 ± 5.0 (9 – 57; N = 9)	40.5 ± 7.2 (13.6 – 78.6; N = 9)	70.2 ± 1.7 (12.8 – 103.4; N = 75)	–	70.6 ± 0.9 (64 – 76; N = 14)	–
<i>Tacca palmata</i>	17.6 ± 1.3 (13 – 23; N = 7)	–	54.5 ± 13.9 (29.4 – 100; N = 5)	85.2 ± 3.1 (12.5 – 225; N = 123)	–	8 (N = 2)	–
<i>Tacca reducta</i>	4.7 ± 0.1 (2 – 9; N = 152)	14.3 ± 0.1 (10 – 18; N = 121)	45.4 ± 4.3 (12.5 – 100; N = 32)	27.7 ± 1.5 (3.7 – 54.8; N = 85)	10184.4 ± 654.0 (7280 – 13140; N = 10)	109.4 ± 2 (99 – 128; N = 16)	93.1
<i>Tacca cristata</i>	9.9 ± 0.4 (4 – 19; N = 67)	14.9 ± 0.4 (10 – 25; N = 53)	35.3 ± 6.0 (7.1 – 80; N = 15)	42.2 ± 3.1 (4.1 – 79.7; N = 48)	10526.0 ± 1351.6 (7670 – 13870; N = 5)	195.8 ± 5.9 (155 – 235; N = 15)	53.8

Nevertheless, all CV values for ovule number were less than 20 %. Yet, CV for the number of seeds ranged from 21.3 to 55.9 for all *Tacca* spp. The overall CV of each species on each floral characteristics was recorded in Table 4.10.

Table 4.10: Coefficient of variance (CV) in percentage for number of flowers, bracteoles, fruits, ovules and seeds of each species (“-” indicated no data for the characteristics of the species).

Species	Flower	Bracteole	Fruit	Ovule	Seed
<i>Tacca bibracteata</i>	29.9	6.1	-	5.4	-
<i>Tacca borneensis</i>	32.5	25.9	50.3	18.8	55.9
<i>Tacca laevis</i>	38.6	17.2	59.4	6.0	48.6
<i>Tacca leontopetaloides</i>	96.5	67.0	80.7	5.0	21.3
<i>Tacca palmata</i>	19.1	-	45.5	-	40.3
<i>Tacca reducta</i>	28.1	10.9	58.3	7.4	49.3
<i>Tacca cristata</i>	32.3	21.9	67.5	11.7	51.2

4.3.4 Pollination ecology

Throughout the observational period, several similar groups of insects were recorded approaching to floral structures of observed *Tacca* species. These insect visitors are ants, grasshoppers, midges, mosquitoes, small beetles, spiders as well as stingless bee. Based on their visitation frequency and visited floral parts, these visitors were categorized into five groups which were midge, stingless bee, ant, mosquito and others (Table 4.11).

Female midges (*Culicoides hinnoi* & *Forcipomyia* spp.: Ceratopogonidae) were the potential pollinators for *Tacca* spp. among all the insect visitors (Appendix B). As identified by Dr Art Borkent, female midges which were collected from *T. borneensis* were *Culicoides hinnoi*, whereas female midges which were collected from other *Tacca* spp. were *Forcipomyia* spp. (Table 4.12). As identified, *T. laevis* had two *Forcipomyia* spp. visited the flowers, whereas only one *Forcipomyia* sp. was captured on flowers of other *Tacca* spp.

Table 4.11: Rate of visitation of different group of insects and their highest number observed per visit. Insects in group “Others” include spider, small beetle, fruit fly and grasshopper; dash (–) indicates that the insect group was not observed approaching the inflorescence of the species.

Species	No. of observation hours (hour)	No. of flowers observed	Rate of insect visitation on flowers					Highest no. of insect recorded per visit				
			Midge	Stingless bee	Ant	Mosquito	Others	Midge	Stingless bee	Ant	Mosquito	Others
<i>T. borneensis</i> (MS)	15	11	0.27	0.18	1.00	0.81	0.09	c. 3	1	2	2	3
(KNP)	20	31	0.26	0.16	1.00	0.68	0.13	c. 15	1	2	3	2
<i>T. laevis</i> (KS)	37	7	1.00	0.14	0.86	0.86	0.30	c. 10	1	2	1	2
(KNP)	28	6	0.67	0.67	0.83	1.00	0.33	c. 5	c. 3	3	1	2
<i>T. leontopetaloides</i>	20	11	1.00	–	0.90	0.36	0.09	c. 20	–	2	4	2
<i>T. palmata</i> (PS)	7	3	0.33	–	1.00	0.33	0.33	c. 4	–	1	1	1
(RRP)	29	6	0.67	–	1.00	0.33	0.17	c. 6	–	1	1	1
<i>T. reducta</i> (KPL)	10	4	0.25	0.50	1.00	1.00	0.50	c. 1	1	3	1	1
(RRP)	73	30	0.63	0.30	0.83	0.87	0.30	c. 5	1	4	1	1
<i>T. cristata</i>	55	15	0.93	–	0.67	0.93	0.60	c. 10	–	4	2	2

Female midges were present in all observed species. Yet, not every flower in anthesis were visited by female midges (Table 4.11). Among all observed flowers, flowers of *T. leontopetaloides* were all visited by female midges. The highest number of female midges flying around the inflorescence of this species was about 20 midges which were the highest record among all species. *Tacca laevis* and *T. cristata* were also visited frequently by female midges. The rate of visitation of female midges to *T. laevis* in KS and KNP were 1.00 and 0.67, respectively, whereas *T. cristata* was 0.93. Both of these species had about 10 female midges visiting during peak insect visitation, except *T. laevis* at KNP (highest number recorded was about five midges). For *T. reducta* and *T. palmata* at RRP, about half (0.63 & 0.67, respectively) of the observed flowers were approached by female midges. At RRP, the maximum number of female midge recorded visiting *T. reducta* was about 5 female midges, whereas for *T. palmata* was about 6 female midges. At PS, *T. palmata* had even lower midge visitation rate (0.33) with highest about four midges at the flowers. At KPL, only 25% of observed flowers of *T. reducta* visited by midges. Lastly, less than 30 % of 42 observed flowers of *T. borneensis* were visited by female midges in both MS and KNP. However, about 15 female midges were recorded as the highest amount of female midges visiting *T. borneensis* at KNP, whereas only about three at MS.

Table 4.12: Identification of midges which visited the inflorescence of each *Tacca* spp.

Species	Identification	No. of identified specimens	Sex
<i>T. borneensis</i>	<i>Culicoides hinnoi</i>	4	Female
<i>T. cristata</i>	<i>Forcipomyia</i> sp. 2	5	Female
<i>T. laevis</i>	<i>Forcipomyia</i> sp. 1	2	Female
	<i>Forcipomyia</i> sp. 2	1	Female
<i>T. leontopetaloides</i>	<i>Forcipomyia</i> sp. 2	1	Female
<i>T. palmata</i>	<i>Forcipomyia</i> sp.	1	Female
<i>T. reducta</i>	<i>Forcipomyia</i> sp. 2	5	Female

Despite their inconsistent visitation, these midges carried pollen grains at the dorsal part of their body while entering and exiting the flowers. In term of accessibility, midges' size is only about 1 mm long and less than 1 mm width (see Appendix B for view under a dissecting microscope). Hence, these tiny midges can access anther and stigma with ease. Moreover, they were attracted to erect flowers which were ongoing anthetic process. Midges were usually spotted during noon between 11:00 to 15:00, whereas for *T. leontopetaloides*, midges were spotted on inflorescence from 15:30 until 02:00. They mostly approached flowers in a very small group which was maximum 2 to 4 flying near the flower at a time (Figure 4.19). Some midges were observed flying around the flowers without landing.

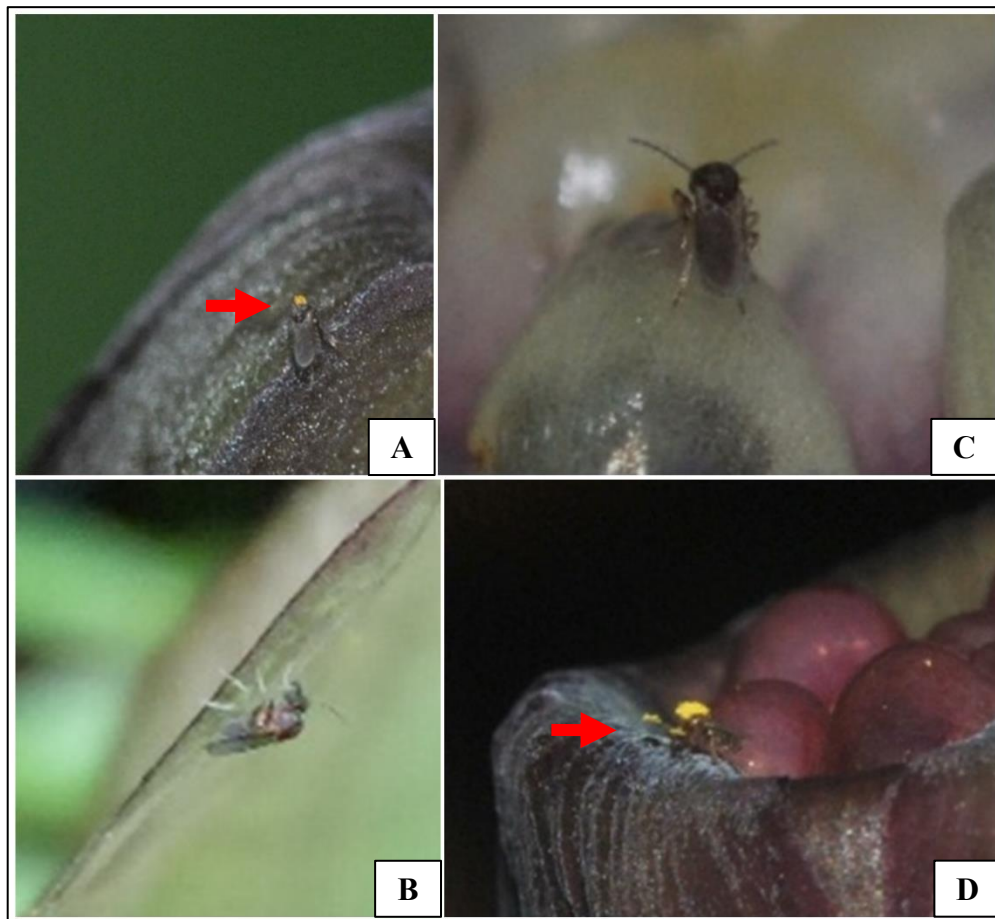


Figure 4.19: *Forcipomyia* spp. on inflorescence of *T. reducta* (A), *T. laevis* (B & C) and *T. cristata* (D) (arrows indicated pollen deposited on midges; see Appendix B for view under dissecting microscope).

Although stingless bee (*Trigona* sp.: Apidae) were known to be collecting pollen from various flowering plants (Heard, 1999), it was seen struggling to access pollen from the anthers of *Tacca*. Its body size rendered it from entering the flowers. The stingless bee was observed trying to reach the anther by its hind leg. Only three *Tacca* spp. (*T. borneensis*, *T. laevis* and *T. reducta*) were approached by stingless bee in all locations. The visitation rate of this insect group was less than midges. For *T. borneensis*, stingless bee was only spotted in 18 % and 16 % of the observed flowers at MS and KNP, respectively. *Tacca reducta* had the rate of visitation by stingless bee equal to 0.5 and 0.3 at KPL and RRP, respectively. Only one bee visited the flowers of both the species whenever it was seen. At KNP, 67 % of the observed *T. laevis*' flowers were visited by stingless bee while 14 % were visited at KS. The maximum number of stingless bee visiting the flower at KNP was about three bees (Figure 4.20).

Next, ant (Formicidae) was one of the insect groups which visited all species of *Tacca*. The visitation rate of ant was high throughout all species. Ant was recorded to be present in all flowers of both *T. borneensis* and *T. palmata* at all location as well as *T. reducta* at KPL. The highest number of ant approached *T. borneensis* and *T. palmata* were two ants and one ant, respectively. *Tacca reducta* at KPL had a maximum of three ants recorded. At RRP, the visitation rate of ants for *T. reducta* was 0.83 with maximum of four ants. *Tacca leontopetaloides* had 0.90 for ant visitation rate with a maximum of two ants visiting a flower at the same time. For *T. laevis*, 86 % and 83 % of the flowers were visited by ants at KS and KNP, respectively. The highest ant number visited *T. laevis* were two ants and three ants at KS and KNP, respectively. *Tacca cristata* from Peninsular Malaysia had slightly lower visitation rate which was 0.67, but there was a maximum of four ants crawling on a flower at the same time. Similar to midges, some ants were tiny enough to enter the gap between

flower structures (Figure 4.21). However, no pollen was deposited on any body part of these ants.



Figure 4.20: *Trigona* sp. (stingless bee) landed on flowers of *T. laevis*.

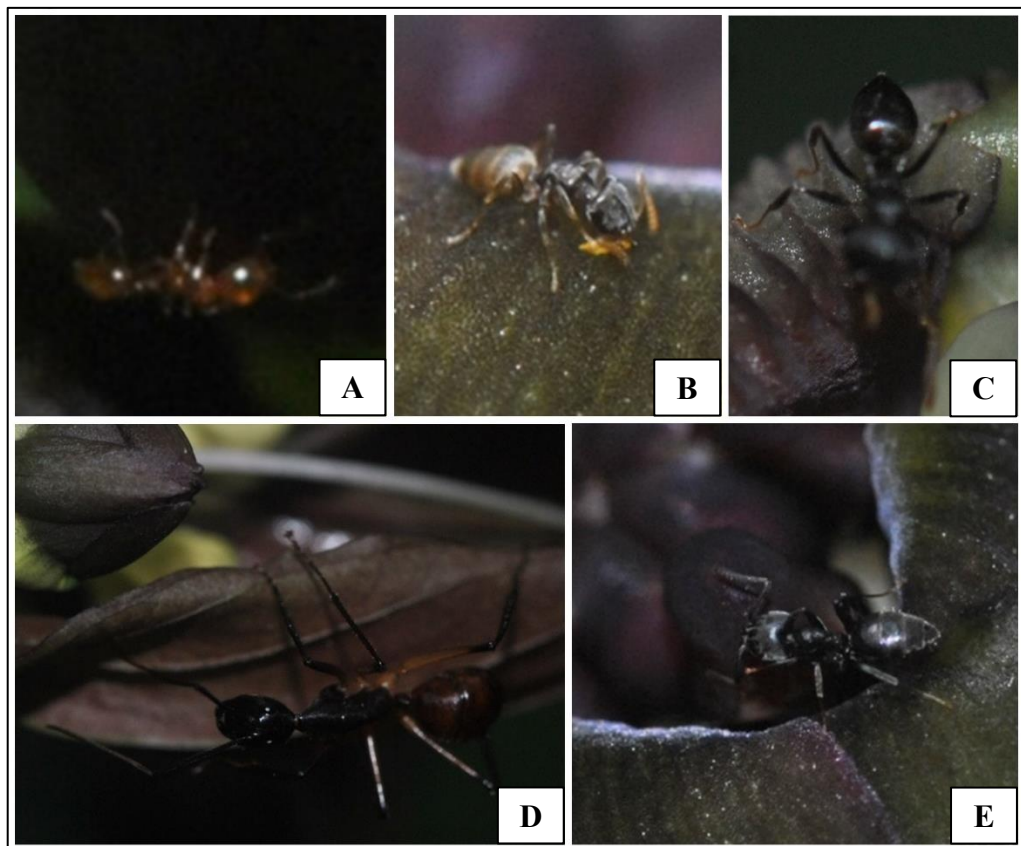


Figure 4.21: Ants visited inflorescences of *T. palmata* (A), *T. reducta* (B & E), *T. laevis* (C & D).

Next, mosquitoes (Culicidae) were also frequently observed flying around the flowers, but mosquitoes are considered large to the gap between anthers and stigma. *Tacca laevis* (KNP) and *T. reducta* (KPL) had all observed flowers visited by one mosquito. Next, *T. borneensis* (MS), *T. cristata*, *T. laevis* (KS) and *T. reducta* (RRP) had considerable high mosquito visitation rate which were 0.81, 0.93, 0.86 and 0.87, respectively. *Tacca borneensis* at KNP had lower mosquito visitation rate (0.68) as compared to MS. Yet, the highest numbers of mosquitoes recorded visiting a flower of *T. borneensis* were two and three mosquitoes at MS and KNP, respectively. *Tacca leontopetaloides* and *T. palmata* had low visitation rate by mosquito with only 36 % and 33 % (both PS and RRP) of the respectively observed flowers were visited. The highest number of mosquito landed on the flower of *T. leontopetaloides* was four mosquitoes. Similar to *T. laevis* and *T. reducta*, only one mosquito was normally spotted visited flower of *T. palmata*. This group of insects was normally seen to be landing on flowers and sucking sap from their succulent parts (Figure 4.22).

The group of other insects consists of insects too large to access the reproductive parts of *Tacca* flowers (Figure 4.23). Their visitation rate was considered low with generally less than 40 % of flowers visited by this group for all species except *T. cristata* (0.6) and *T. reducta* (0.5 at KPL). The highest number of other insects was ranged from one to three insects approaching a flower at a time. Within this group, grasshopper (Orthoptera) was the most frequently observed insect. Grasshoppers were only seen destroying the floral parts by chewing any succulent part including the stigma structure and anthers. This florivore was excluded as the potential pollinator. Another group of the visitor which was not able to access the reproductive parts was spider (Araneae). Most of the time, spiders were found building nest on bracts to prey on other insects which approached the inflorescence. Based on my observation, small beetles and fruit flies were least observed on an inflorescence. Beetles

(Coleoptera) only stopped at either bracts or bracteoles which also did not aid in pollinating the flowers. Fruit flies (*Drosophila*: Diptera) were observed on flowers of *T. palmata*, but the role remained unclear because they were not able to access the reproductive parts.



Figure 4.22: Mosquito landed on flowers of *T. laevis* (A) and *T. reducta* (B).

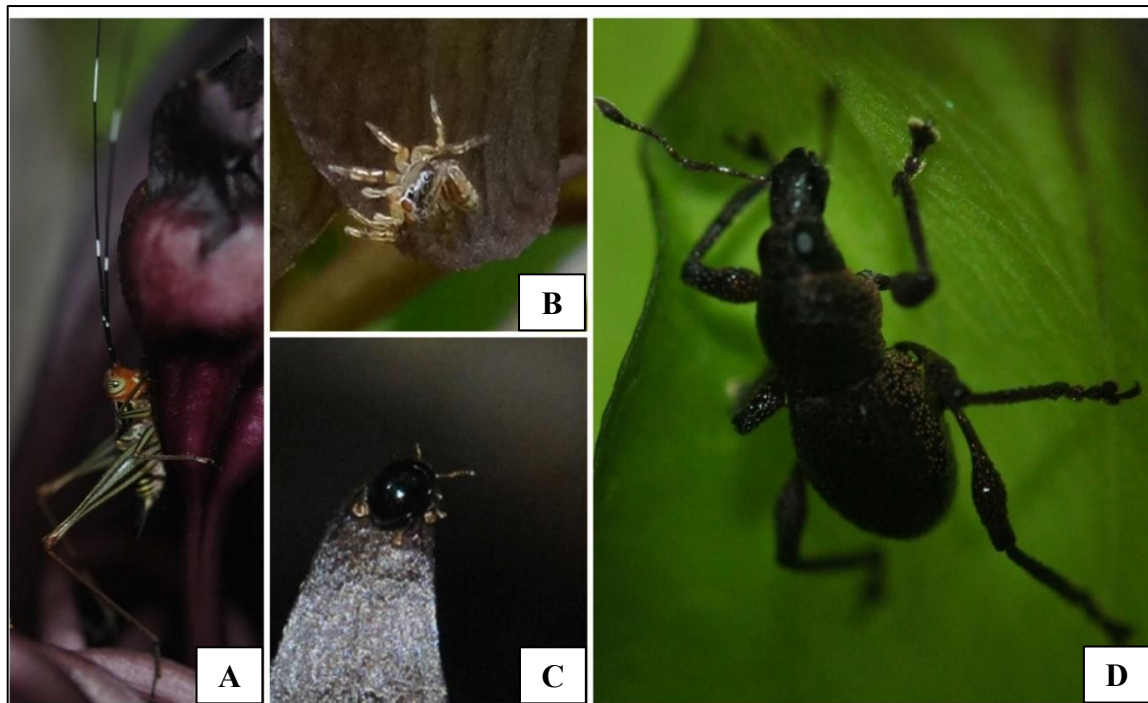


Figure 4.23: Other insect visitors (A: grasshopper on *T. borneensis*; B: spider on *T. reducta*; C & D: beetles on *T. reducta* and *T. leontopetaloides*, respectively) of inflorescences of *Tacca*.

For *T. cristata*, another type of fly, known as fungus gnat (Sciaridae), was observed. Rate of visitation of this fungus gnat is 0.73 with two fungus gnats recorded as the highest number per visit. They were not able to differentiate anthetic and post-anthetic flowers, and too large (c. 2 x 1 mm) to enter the flower to help pollination. The larvae were frequently found burrowed into flowers and destroy the ovules or the developing seeds. Sometimes, they were found caused necrosis of the inflorescence bud. Inflorescence of *T. cristata* acts as oviposition site for fungus gnat, but the specific location of eggs on flower or inflorescence was unknown (Figure 4.24). Out of 67 inflorescences, 23 inflorescences were lost, likely due to the infestation of the fungus gnat. Moreover, six fruits of three infructescences were found to be completely emptied (no seed), while several fruits partially damaged (Figure 4.24D).



Figure 4.24: A: Fungus gnats visited post-anthetic flowers. B: Larvae of fungus gnats infested inflorescence bud. C: Larvae of fungus gnats found within the dissected flower. D: Fruit with seed loss due to the infestation of fungus gnat's larvae.

Different groups of insect visited various parts of the inflorescence. First, midges were observed to be a frequent visitor for anthetic flowers for all species, but not in all species for post-anthetic flowers. The midges which landed on the flower in post-anthesis phase did not move to the reproductive parts. Midges also stopped at bracts and bracteoles (if present) in all *Tacca* spp. Next insect group stingless bee was also mostly approached anthetic and post anthetic flowers of all visited species, and rarely landed on the bracts (only seen in *T. laevis*). It seems like stingless bee was unable to distinguish both phases of the flowers. In contrast to other insect groups, ant was recorded to be visiting all floral parts when it was present. Small ant was observed entering the flower which may contact the reproductive parts of the flower. However, ant was never spotted with pollen deposition on any body parts. Similar to stingless bee, mosquito was seen landed on both anthetic and post anthetic flowers, rarely on bracts (only seen in *T. borneensis*). Mosquito seemed to be more interested in the succulent flower parts such as stigmatic lobes and stamen caps instead of the reproductive parts.

In the group of other insects, spider and beetle, if present, were always located on bracts or bracteoles. Whereas for grasshopper, it chewed on all floral parts. Thus, it did not discriminate any parts of the inflorescence. Fruit fly, which was not a common visitor, usually landed on perianths of flower. Table 4.13 indicated the insect visitors and the floral parts they landed during their visitation.

Fungus gnats which only visited *T. cristata* were found to crawl on flowers regardless of the anthesis stage. Occasionally, fungus gnats were observed to land on bracts before approaching the flowers (Table 4.14).

Table 4.13: Floral parts of *Tacca* spp. visited by insect.

Species	Floral parts	Insect visitors				
		Midge	Stingless bee	Ant	Mosquito	Others
<i>T. borneensis</i>	Flowers	Anthesis	/	/	/	/
		Post-anthesis	-	/	/	-
	Bracts	/	-	/	/	/
	Bracteoles	/	-	/	-	/
<i>T. laevis</i>	Flowers	Anthesis	/	/	/	-
		Post-anthesis	-	/	/	/
	Bracts	/	/	/	-	/
	Bracteoles	/	-	/	-	/
<i>T. leontopetaloides</i>	Flowers	Anthesis	/	-	/	-
		Post-anthesis	-	-	/	-
	Bracts	/	-	/	/	/
	Bracteoles	/	-	/	-	-
<i>T. palmata</i>	Flowers	Anthesis	/	-	/	/
		Post-anthesis	/	-	/	/
	Bracts	/	-	/	-	/
<i>T. reducta</i>	Flowers	Anthesis	/	/	/	/
		Post-anthesis	-	-	/	/
	Bracts	-	-	/	-	/
	Bracteoles	/	-	/	/	/

Table 4.14: Floral parts of *T. cristata* visited by insect.

Species	Floral parts	Insect visitors					
		Midge	Stingless bee	Ant	Mosquito	Fungus gnat	Others
<i>T. cristata</i>	Flower	Anthesis	/	-	/	/	-
		Post-anthesis	/	-	/	-	-
	Bracts	/	-	/	-	/	/
	Bracteoles	/	-	/	-	-	/

4.3.5 Field Manipulation Experiment

4.3.5.1 *Tacca borneensis*

For the control of *T. borneensis*, 16 out of 26 sampled inflorescences yielded fruits. This gave rise to 61.5% of the fruiting percentage. As for the removal of the bracts and bracteoles, all three inflorescences of *T. borneensis* did not produce any fruit. Low number of inflorescence for treatment was sampled due to the insufficient number of inflorescence developed during the sampling period.

4.3.5.2 *Tacca laevis*

For the control of *T. laevis*, out of 24 sampled inflorescences, 17 inflorescences developed into infructescences with fruits (fruiting percentage = 70.8 %), whereas for inflorescence without bracts and bracteoles, four infructescences were produced out of six inflorescences (fruiting percentage = 66.7 %).

For *T. laevis*, fruit set percentage for control ranged from 12.5 % to 100 %, whereas for treatment ranged from 33.3 % to 80 %. Mean number of fruit set percentage for control and treatment were $81.6 \% \pm 6.8$ ($N = 17$) and $58.3 \% \pm 12.6$ ($N = 4$), respectively. Removing the bracts and bracteoles did not show any significant effect (Welch $t = 1.62$, $df = 5$, $p = 0.1659$; $t_{crit} = 2.57$, $\alpha = 0.05$) on fruit set (Table 4.15). Next, seed set percentage for control ranged from 3.9% to 94.4%, whereas for treatment ranged from 7.9% to 78%. Average seed number percentage for control and treatment were $37.3\% \pm 1.6$ ($N = 135$) and $46.4\% \pm 4.7$ ($N = 16$), respectively. Seed sets of both control and removal of bracts and bracteoles did not show significant difference (Welch $t = -1.85$, $df = 18$, $p = 0.07971$; $t_{crit} = 2.10$, $\alpha = 0.05$). Hence, both fruit set and seed set that yielded from normal inflorescences and inflorescences

without bracts and bracteoles did not show any significant differences at 95 % confidence level.

Table 4.15: Fruiting percentage, mean, range and number of replicates of fruit number, fruit set, seed number and seed set of control and treatment for *T. laevis*.

Treatment	Fruiting percentage (%)	Average number of fruits	Average fruit set (%)	Average number of seeds	Average seed set (%)
Control	70.8 (N = 24)	4.65 ± 0.7 (1 – 12, N = 17)	81.6 ± 6.8 (12.5 – 100, N = 17)	56.9 ± 2.4 (6 – 144, N = 135)	37.3 ± 1.6 (3.9 – 94.4, N = 135)
Removal of bracts and bracteoles	66.7 (N = 6)	4.25 ± 1.3 (2 – 8, N = 4)	58.3 ± 12.6 (33.3 – 80, N = 4)	70.8 ± 7.1 (12 – 119, N = 16)	46.4 ± 4.7 (7.9 – 78, N = 16)

4.3.5.3 *Tacca reducta*

For the control of *T. reducta*, out of 45 sampled inflorescences, 32 inflorescences developed into infructescences (fruiting percentage = 71.1 %), whereas for inflorescence with bracts and bracteoles removed, 13 inflorescences developed into infructescences out of 25 inflorescences (fruiting percentage = 52 %).

For *T. reducta*, removing the inflorescence bracts and bracteoles significantly affect the fruit set (Welch $t = 2.25$, $df = 42$, $p = 0.03$; $t_{crit} = 2.02$, $\alpha = 0.05$) and seed set (Welch $t = 2.42$, $df = 24$, $p = 0.023453$; $t_{crit} = 2.07$, $\alpha = 0.05$) at 95 % confidence level (Table 4.16). Fruit set percentage per inflorescence for control ranged from 12.5 % to 100 %, whereas for treatment ranged from 16.7 % to 50 %. Mean number of fruit set percentage per inflorescence for control and treatment were $45.4 \% \pm 4.3$ (N = 32) and $33.5 \% \pm 3.1$ (N = 13), respectively. Next, seed set percentage for control ranged from 3.7 % to 54.8 %, whereas for treatment ranged from 1.8 % to 53.9 %. Average seed number percentage for control and treatment

were $27.7 \% \pm 1.5$ (N = 85) and $18.6 \% \pm 3.5$ (N = 18), respectively. Hence, the fruit and seed set developed from normal inflorescence was significantly more than the one produced by inflorescence without bracts and bracteoles.

Table 4.16: Fruiting percentage, mean, range and number of replicates of fruit number, fruit set, seed number and seed set of control and treatment for *T. reducta*.

Treatment	Fruiting percentage (%)	Average number of fruits	Average fruit set (%)	Average number of seeds	Average seed set (%)
Control	71.1% (N = 45)	2.10 ± 0.23 (1 – 5, N = 30)	45.4 ± 4.3 (12.5 – 100, N = 32)	30.31 ± 1.62 (4 – 60, N = 85)	27.7 ± 1.5 (3.7 – 54.8, N = 85)
Removal of bracts and bracteoles	52.0% (N = 25)	1.38 ± 0.14 (1 – 2, N = 13)	33.5 ± 3.1 (16.7 – 50, N = 13)	20.33 ± 3.79 (2 – 59, N = 18)	18.6 ± 3.5 (1.8 – 53.9, N = 18)

4.3.5.4 *Tacca cristata*

For control of *T. cristata*, out of 30 sampled inflorescence, 15 had developed into infructescence with fruits (fruiting percentage = 50 %), whereas for inflorescence without bracts and bracteoles, 5 infructescences produced out of 13 sampled inflorescence (fruiting percentage = 38.5 %).

Fruit set percentage for control ranged from 7.1 % to 80 %, whereas for treatment ranged from 9.1 % to 66.7 %. Mean number of fruit set percentage for control and treatment were $35.3 \% \pm 6.0$ (N = 15) and $35.0 \% \pm 9.4$ (N = 5), respectively. Removing bracts and bracteoles did not show any significant different (Welch t = 0.0263, df = 8, p = 0.97966; t_{crit} value = 2.37, $\alpha = 0.05$) effect on fruit set. Next, seed set percentage for control ranged from 4.1% to 79.7%, whereas for treatment ranged from 12.3 % to 88.9 %. Average seed number percentage for control and treatment were $42.2 \% \pm 3.1$ (N = 48) and $48.0 \% \pm 6.1$ (N = 15),

respectively. The seed set from both control and removal of bracts and bracteoles also did not show any significant difference (Welch $t = -0.865$, $df = 22$, $p = 0.39644$; t_{crit} value = 2.07, $\alpha = 0.05$). Table 4.17 summarized the data analysis for fruit set and seed set of both control and treatment of field manipulation experiment for *T. cristata*.

Table 4.17: Fruiting percentage, mean, range and number of replicates of fruit number, fruit set, seed number and seed set of control and treatment for *T. cristata*.

Treatment	Fruiting percentage (%)	Average number of fruits	Average fruit set (%)	Average number of seeds	Average seed set (%)
Control	50.0 (N = 30)	3.73 ± 0.7 (1 – 9, N = 15)	35.3 ± 6.0 (7.1 – 80, N = 15)	82.5 ± 6.1 (8 – 156, N = 48)	42.2 ± 3.1 (4.1 – 79.7, N = 48)
Removal of bracts and bracteoles	38.5 (N = 13)	3.0 ± 0.6 (1 – 4, N = 5)	35.0 ± 9.4 (9.1 – 66.7, N = 5)	94.1 ± 11.8 (24 – 174, N = 15)	48.0 ± 6.1 (12.3 – 88.9, N = 15)

4.3.5.5 Interspecific comparison on data of field manipulation experiment

Fruit set of different species varied (Table 4.18), but the fruit sets of treatments for all species were generally less than control (Figure 4.25). The mean values of all fruit set for control of all three species were greater than the one with bracts and bracteoles removed. In contrast, seed set for every species showed different relationship for control and treatments (Figure 4.26). For *T. laevis* and *T. cristata*, most seed sets of control distributed at a lower percentage than treatment. Thus, the average seed sets of treatment for both species were higher than control. Whereas for *T. reducta*, seed set developed from inflorescence without bracts and bracteoles was mostly in a lower percentage than control.

Table 4.18: Comparing all species fruit set and seed set of treatment removal of bracts and bracteoles.

Species		Average fruit set (%)	Average seed set (%)
<i>T. laevis</i>	Control	81.6 ± 6.8 (12.5 – 100, N = 17)	37.3 ± 1.6 (3.9 – 94.4, N = 135)
	Treatment	58.3 ± 12.6 (33.3 – 80, N = 4)	46.4 ± 4.7 (7.9 – 78, N = 16)
<i>T. reducta</i>	Control	45.4 ± 4.3 (12.5–100, N=32)	27.7 ± 1.5 (3.7–54.8, N=85)
	Treatment	33.5 ± 3.1 (16.7–50, N=13)	18.6 ± 3.5 (1.8–53.9, N=18)
<i>T. cristata</i>	Control	35.3 ± 6.0 (7.1 – 80, N = 15)	42.2 ± 3.1 (4.1 – 79.7, N = 48)
	Treatment	35.0 ± 9.4 (9.1 – 66.7, N = 5)	48.0 ± 6.1 (12.3 – 88.9, N = 15)

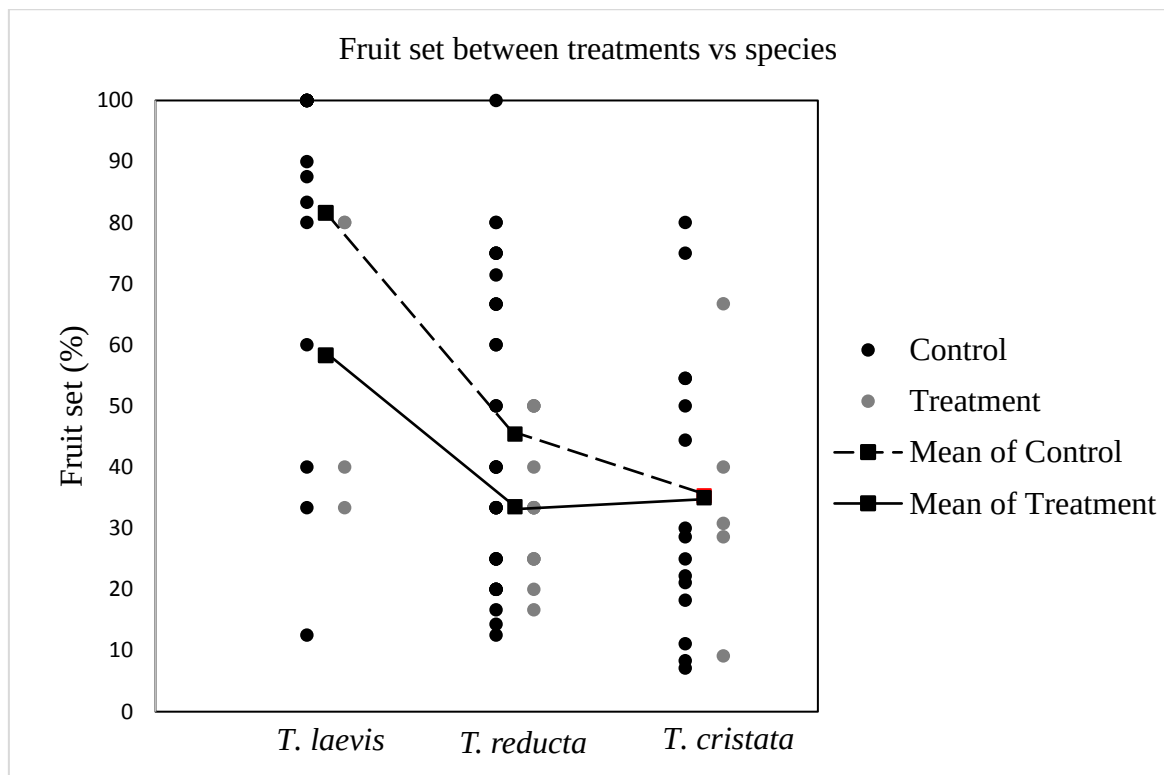


Figure 4.25: Graph of fruit set against species for both control and treatment. (Dash line indicated mean of control).

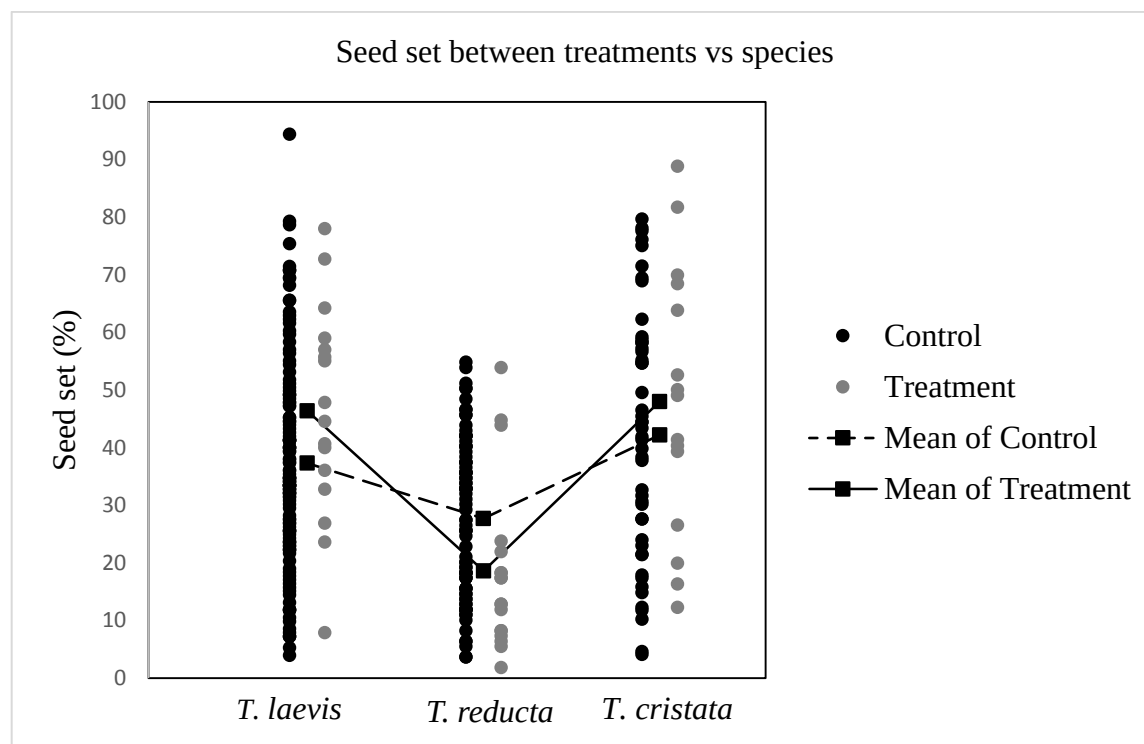


Figure 4.26: Graph of seed set against species for both control and treatment. (Dash line indicated mean of control).

4.3.6 Controlled Pollination Experiment

4.3.6.1 *Tacca borneensis*

Artificial self-pollination produced fruits with seeds ranged from seven to 24 seeds. The mean value for number of seeds is 15.5 ± 8.5 ($N = 2$). The average seed set for artificial self-pollination is $6.5 \% \pm 3.6$ ($N = 2$) with minimum value 2.9 % and up to 10.1 % (Table 4.19). All fruits of artificial self-pollination suffered from early abortion in which the fruit stalk began to rot with other unfertilized flower before the fruit ripened. Autogamous self-pollination did not have any fruit set.

Table 4.19: The seed set data on controlled pollination experiment of *T. borneensis*.

Treatment	Average seed number	Average seed set (%)
Control	131.2 ± 6.8 (9 – 305; N = 117)	55.1 ± 2.8 (3.8 – 128; N = 117)
Artificial self-pollination	15.5 ± 8.5 (7 – 24; N = 2)	6.5 ± 3.6 (2.9 – 10.1; N = 2)
Artificial cross-pollination	104	43.7

4.3.6.2 *Tacca laevis*

The seed number of control had a mean value equal to 56.9 ± 2.4 (N = 135). The range of seed number was six to 144 seeds. For seed set, the mean value was 37.3 ± 1.6 (N = 135), which range from 3.9 % to 94.4 % (Table 4.20). Artificial self-pollination produced fruits with seeds ranged from eight to 30 seeds. The mean value for the number of seeds is 17.8 ± 4.2 (N = 5). The average seed set for artificial self-pollination is $11.7 \% \pm 2.7$ (N = 5) with minimum value of 5.2 % and up to 19.7 %. For cross-pollination, the average seed number and seed set were the lowest with mean of 8.3 ± 5.9 and $5.5 \% \pm 3.9$, respectively. The seed number ranged from one to 20 seeds, and the percentage ranged from 0.7 % to 13.1 % only. Autogamous self-pollination did not have any fruit set.

Table 4.20: The seed set data on controlled pollination experiment of *T. laevis*.

Treatment	Average seed number	Average seed set (%)
Control	56.9 ± 2.4 (6 – 144; N = 135)	37.3 ± 1.6 (3.9 – 94.4; N = 135)
Artificial self-pollination	17.8 ± 4.2 (8– 30; N = 5)	11.7 ± 2.7 (5.2 – 19.7; N = 5)
Artificial cross-pollination	8.3 ± 5.9 (1 – 20; N = 3)	5.5 ± 3.9 (0.7 – 13.1; N = 3)

Calculated F value from Welch ANOVA ($F = 46$, $df = 5$, $p = 0.0007054$) was more than critical F value ($F_{crit} = 5.79$, $\alpha = 0.05$) at 95 % confidence level. As a result, the null

hypothesis was rejected, the mean values of treatments were significantly different. The post-hoc test showed that average seed set from control was significantly larger than both artificial pollinations, while no difference between the artificial pollination tests.

4.3.6.3 *Tacca reducta*

For *T. reducta*, average seed set for control was 30.3 ± 1.6 (N = 85) with range four to 60 seeds. The mean value of seed set was 27.7 ± 1.5 (N = 85). The range of the seed set was from 3.7 % to 54.8 %. The average number of seed produced by artificial self-pollination was 25.8 ± 2.8 (N = 13). The fruit which developed from artificial self-pollination produced 11 to 43 seeds. The range of seed set for this test was 10.1 % to 39.3 %, with mean value of $23.62 \% \pm 2.53$ (N = 13). Artificial cross-pollination test for *T. reducta* had mean seed value equal to 21.7 ± 9.3 (N = 3) with range 10 to 40 seeds. The seed set ranged from 9.1 % to 36.6 %, with mean value $19.80 \% \pm 8.5$ (N = 3). Table 4.21 shown the comparison between the average of seed number and seed set of *T. reducta* among the treatments of controlled pollination test. Autogamous self-pollination did not have any fruit set.

Table 4.21: The seed set data on controlled pollination experiment of *T. reducta*.

Treatment	Average seed number	Average seed set (%)
Control	30.3 ± 1.6 (4 – 60; N = 85)	27.7 ± 1.5 (3.7 – 54.8; N = 85)
Artificial self-pollination	25.8 ± 2.8 (11 – 43; N = 13)	23.6 ± 2.5 (10.1 – 39.3; N = 13)
Artificial cross-pollination	21.7 ± 9.3 (10 – 40; N = 3)	19.8 ± 8.5 (9.1 – 36.6; N = 3)

Welch ANOVA on treatments yielded $F(2, 5) = 1.143$, $p = 0.389$. The calculated F value was less than F_{crit} value which is 5.79 at $\alpha = 0.05$. Thus, the null hypothesis was accepted, in which mean values of all the treatment did not have any significant difference.

4.4 Discussion

4.4.1 Floral mechanism

The flowering period of *Tacca* spp. can be categorized into three groups. The first group consisted of *T. leontopetaloides* which bloomed on an annual basis. All inflorescences with an abundant number of flowers per inflorescence (up to 145 flowers) bloomed at a specific period (probably starting from July to early September). The second group included *T. borneensis* and *T. palmata* which bloomed sporadically (*T. borneensis*: six inflorescences in April, and less than four inflorescences in other months; *T. palmata*: either no inflorescence or one inflorescence at a time). Inflorescences of these species also developed a large number of flowers per inflorescence (*T. borneensis*: up to 25 flowers; *T. palmata*: up to 23 flowers), but not all plant individuals bloomed at the same period. The third group included *T. cristata*, *T. laevis* and *T. reducta* which bloomed throughout the year. These species had inflorescence with a lower number of flowers per inflorescence (*T. cristata*: up to 19 flowers; *T. laevis*: up to 12 flowers; *T. reducta*: up to 9 flowers).

Inflorescences of all species had flowering sequence starting from the middle-most flowers in day one and followed by the adjacent flowers on the next day until the outer most flowers on the last day. All the observed species started anthesis in the morning (5.40 a.m. – 7.40 a.m.), except *T. leontopetaloides* which its flowers bloomed in late evening (5.40 p.m.). Despite that, the anthesis period of *Tacca* spp. lasted for approximately 10 to 12 hours. Within this period, the floral movements recorded were perianths opening till flower declinate. A faint floral scent was only detected from *T. laevis* and *T. leontopetaloides*. The stigmas of flower buds for all species were observed with sticky droplets. Pollen extrusion for *T. borneensis*, *T. laevis* and *T. reducta* was about one to three hours after the outer

perianth reflexed. This result is not similar to the previous study on *T. chantrieri* in which the pollen was recorded to extrude before the flower opened (Zhang et al., 2005).

Next, the number of flowers bloomed on each day per inflorescence varied among species. For *T. cristata*, *T. laevis*, *T. palmata* and *T. reducta*, only one or two (or three) flowers bloomed per day, whereas *T. borneensis* and *T. leontopetaloides* were recorded to have more than 10 flowers per inflorescence blooming at the same day. This situation increases the occurrence of geitonogamy. Flowers of *T. leontopetaloides* and *T. palmata* had perianths incompletely reflexed during anthesis. The perianths only partially opened with slight gaps in between perianths. At the end of anthesis, the perianths joined again, covering the anthers and stigma. For other species, the perianths remained opened after the flowers bloomed.

There are two types of infructescences in *Tacca* spp., one with the peduncle twisted at the end of anthesis, another one with peduncle remained standing after the anthesis ended. The earlier included *T. borneensis*, *T. cristata*, *T. laevis* and *T. reducta*, and the later included *T. leontopetaloides* and *T. palmata*. The first group develops fruits with obpyramidal shaped with the bracts and bracteoles not persistent, whereas the second group develops round fruits with persistent floral structures. For *T. leontopetaloides*, white testa was found covering the seed in the fruit. This structure was absent in all other observed species. Whereas for fruit colour, fruits of *T. palmata* turned from green to red when ripe, fruits of *T. leontopetaloides* turned from green to yellow when ripe, fruits of *T. laevis* remained green when ripe, and lastly *T. borneensis*, *T. cristata* and *T. reducta* remained dark purple when ripe. These variations in fruit position and colour probably relate to their seed dispersal mechanism.

Based on the floral characteristics, the number of flowers per inflorescence of each species had different mean values. The average numbers of bracteoles per inflorescence for *T.*

bibracteata, *T. reducta* and *T. cristata* were similar. However, their CV in bracteoles number were all different. Moreover, the CV in the number of flowers in every species did not have any obvious relation with their CV in bracteoles. Thus, the number of bracteoles may not depend on the number of flowers per inflorescence.

The CV of seed number increased as the CV of ovule number increased. This relation implies that *Tacca* spp. produced a higher number of ovules as a response to stochastic pollen receipt among flower (Burd, 1995). Species with this adaptation adopted a bet hedging strategy to encounter the random fertilization success (Knight et al., 2005). In addition, the intraspecific fruit number also showed a considerable high variation as some inflorescences received more frequent pollinator visitation than the other inflorescences of the same species.

For pollen of *Tacca* spp., the mean values for the pollen amount are almost similar between species. But, the number of ovules highly differed between *Tacca* spp. As a result, the P:O ratio in observed *Tacca* spp. varied. Similar to *T. chantrieri*, the ratio fall within the range of P:O ratio of facultative self-pollinated species (Zhang et al., 2005). This group of species was characterized by their self-compatibility and able to self-pollinated before or during flower blooming although pollinators may visit the flower afterward (Cruden, 1977).

4.4.2 Floral visitors

For *Tacca* spp., the pollinators had to be tiny to enter the flowers without destroying any floral structure. It is because the stigma is located under the umbrella-like gynoecium structure. Furthermore, the gap between androecium and gynoecium is small. This small gap acts as a barrier for screening out insect which collects pollens and thus fertilizes the flower. Out of all the insect visitors, only midges were able to pass through the gaps. Smaller than

the size of a pin-head, this tiny creature can enter through the little gaps of *Tacca* flowers at ease to reach the reproductive parts of the flower. Pollen was found deposited on the dorsal area of some midges when they visited and left the flowers. Although midges were observed flying into the flowers, their movements within the flowers remained unclear. Whether these midges visited anther or stigma first were not known. Both male and female anthesis of *Tacca* flowers timed in an overlapped period. In addition, the position of anthers and stigma were adjacent, and thus promoting the possibility of self-pollination. Autonomous self-pollination did not bear any result proving that these species are incapable of transferring own pollen to stigma without external aids.

Midges, and other common names such as fungus gnats and sand flies, represented a collective name of genera of the family Ceratopogonidae (Diptera) (Hill & MacDonald, 2010). The moist surrounding is an essential habitat of midges as the larvae stage of most species is restricted to semiaquatic and aquatic environments (Borkent, 2004). While some species can cause serious, irritating pest problems, midges (*Forcipomyia hardyi*) were known to be an important pollinator of cacao flowers (O'Doherty & Zoll, 2012). Female midges which visited *Tacca* flowers were identified belonging to genus *Forcipomyia* and *Culicoides*. *Culicoides* spp. were also identified as a pollinator of *Arum* spp. which had no noticeable floral scent (Gibernau et al., 2004). Not only *Tacca* spp. in Sarawak, *T. cristata* at FRIM in Peninsular Malaysia also observed to have visitation of *Forcipomyia* midges. These midges were the same species as the midges collected from Sarawak. In addition, unidentified female midges also had been reported to approach *T. cristata* in Singapore (Lim & Raguso, 2017). However, the mechanism adopted by the inflorescence of *Tacca* spp. to attract only female *Forcipomyia* midges was not known. Floral scent or compound which is perceptible to midges but not human may be emitted by *Tacca* spp. Other than that, other

attractants may also be involved such as visual cues, carbon dioxide emission, heat production or humidity (Gibernau, 2004; Jarau et al., 2010; Liu et al., 2009). These hypotheses require further field evaluations to confirm.

Mass reproduction of midges maintains the population of midges in the field, which ensure the higher success rate of pollination on *Tacca* flowers. In addition, the length of the life cycle of midges depends on food availability and ambient temperature (Hill & MacDonald, 2010). Varying from 3 weeks up to more than a year, midges reach their reproductive maturity (Hill & MacDonald, 2010). Short generation time benefits pollination as the increase in population size of pollinator raises the chance of floral visitation. Whereas, long generation time sustains the pollinator population size throughout a year.

Another *Tacca* species, namely *T. chantrieri* in Southwest China was reported capable of autonomous selfing (Zhang et al., 2005). Insect visitation was stated to be scarce on *T. chantrieri*, in contrast with this study, only *Trigona* stingless bee was suspected to be the potential pollinator among all the insect visitors (Zhang et al., 2005). In West Sarawak, this bee was also spotted visiting the flowers. Due to its body size, stingless bee may collect the pollens under the hooded shaped anthers using its hind leg, but its effectiveness in pollinating the flowers was uncertain. Observations on *T. chantrieri* at other locations are vital to confirm whether the absence of midge in attending this flower is because of spatial or temporal variation in the midge population. Yet, molecular evidence showed this species had significant quantities of seeds produced from self-fertilization in the natural population (Zhang et al., 2005). In addition, the population of *T. cristata* in Seremban, Malaysia was also reported to be highly genetically homogenous (Zhang et al., 2006). Neighbouring distance of anthers and stigmas, anther dehiscence before flower opening, and bending motion of pedicel at the end of anthesis increased the selfing rates of *T. chantrieri* (Zhang et

al., 2005). *Tacca borneensis*, *T. cristata*, *T. laevis* and *T. reducta* also acquired these morphologies, except dehiscence of anther before flower opening. However, autonomous self-pollination did not happen in all these observed species.

Observation findings of this study coincided with observations of Lim & Raguso (2017) on *T. cristata*. First, midges were present during anthesis with yellowish pollen on their dorsal part. Second, midges only visited blooming flowers which are erected, instead of pendent flowers or buds. Third, midge visitation was sporadic, mostly during daytime hours. Among the observed species, *T. borneensis*, *T. palmata* and *T. reducta* had rather low midges' visitation rate especially *T. borneensis*. *Tacca borneensis* bloomed more than 10 flowers per day. The absence of midges' visitation per day will greatly lower the visitation rate. Or, the low visitation rate of midges may be due to spatial variation as KNP and MS probably had a low population of midges. This situation was also observed in *T. reducta* and *T. palmata* at RRP. Interspecific competition on pollinator was less likely to occur as both of the species received a more or less equal amount of visitation rate even though they are near to each other. Moreover, *T. palmata* bloomed less frequent than *T. reducta*. And lastly, florivores' attacks on *Tacca* flowers were frequently observed. Figure 4.27 depicted the damages caused by florivores on inflorescences and herbivores on fruits of *Tacca* spp. These destructions caused a loss in reproductive chances of *Tacca* spp.

As mentioned in Lim & Raguso (2017), some inflorescences were visited by significantly more midges than others. In West Sarawak, some flowers were not visited by any midges throughout anthesis during observation. There are several common reasons for insect visitors attracted to flowers such as food rewards (Nepi et al., 2018) and breeding sites (Sakai, 2002). However, *Tacca* does not offer nectar. Post anthetic flowers of *Tacca* spp. in Sarawak were checked, but no larvae of midges were detected. *Tacca cristata* was found to have Sciaridae

flies oviposited on flowers, but this insect did not help in pollination. No reward was offered to midges in return for their visits. Hence, active pollination can be excluded. Due to the lack of evidence of a mutualistic relationship, deceptive pollination mechanism was probably developed by *Tacca* flowers to attract midges for pollination purpose. However, the type of attraction utilized by the flowers remained not known. Insect behaviour of female midge was required to be studied to understand the reason of its floral visitation.



Figure 4.27: Flowers and immature fruits of *T. laevis* (A) and *T. reducta* (B & C) were attacked by florivores.

4.4.3 Role of bracts and bracteoles

Removal of bracts and bracteoles had a different degree of effect on fruiting of different *Tacca* spp. First, fruit development was completely hindered in *T. borneensis* when the bracts and bracteoles were removed. Other species had decreased amount of inflorescences developed into infructescences when the floral structures were absent. This reduction indicated that the reproduction success was affected, although the differences in the percentage of fruiting between normal inflorescences and inflorescences without bracts and bracteoles were low among these species (4.1 % – 19.1 %). This result can be caused by two reasons. One, the absence of floral structures lower the attractiveness of *Tacca*'s inflorescence toward pollinators. Two, mechanical damage caused by cutting off the floral structures deteriorated the development of inflorescence as the injured area was susceptible to any infection.

Next, among the infructescences, the mean values of fruit sets between control and treatment in all field manipulation study of each species did not show any significant differences, except *T. reducta*. *Tacca reducta* had smaller bracts, compared to *T. cristata* and *T. laevis*. Thus, this result showed a conflict of these floral structures being attractive to pollinators. Removal of bigger bracts (*T. cristata* and *T. laevis*) showed no significant difference in reproductive success, but the removal of smaller bracts (*T. reducta*) reduced the fruit set. One of the explanations for this conflict was related to the number of days the inflorescence visited by pollinators. As fruit set estimated the percentage of flowers fertilized per inflorescence, the number of fruits produced was equivalent to the number of days the inflorescence received pollinator visitation. Inflorescences of *T. laevis* and *T. reducta* generally had one or two flowers bloomed per day, whereas inflorescence of *T. cristata* generally had one to three flowers bloomed per day. However, *T. reducta* had overall fewer

flowers per inflorescence than the other two species, which means shorter flowering duration. In addition, the locality of RRP may have a smaller population of midges (potential pollinators) as mentioned in the earlier section.

Third, the seed set data produced a rather unexpected result for *T. cristata* and *T. laevis*. Although the seed set analysis showed no differences in field manipulation experiment, the control of both of the species yielded fruits with a less average number of seeds than inflorescence without bracts and bracteoles. Seed set was mainly influenced by resource allocation, pollen deposition and ovule quantity (Burd, 1995; Strelin & Aizen, 2018). Assuming the number of ovules did not vary significantly within species (as CV less than 12%), either pollination or plant resources limit seed set. Supposedly, the fruit set between control and treatment did not differ significantly. Thus, the resource allocated for fruit production in every infructescence should be similar. Hence, the unpredictable pollinator visitation may be the main cause for variation in seed set. For further study, the pollen count on stigma is necessary to determine whether pollen limitation or excess pollen deposition is the reason behind low seed to ovule ratios. And, whether there is a difference in the pollen count on the stigma of flowers of inflorescence with or without these floral structures.

To conclude, the data in this study were limited to link any role of bracts and bracteoles on reproductive success. The result of field manipulation experiment for *T. cristata* and *T. laevis* were similar to the one conducted by Zhang et al. (2005). However, *T. borneensis* fruit production was severely impeded. *Tacca reducta* had reduced fruit and seed set when bracts and bracteoles were removed. Hence, the role of bracts and bracteoles maybe different between species. The function of these floral structures could be examined through other aspects such as scent emission in addition to visually attractive.

4.4.4 Self- and cross-pollination

The reproductive success in controlled pollination experiment of *T. laevis* and *T. reducta* indicated that these two species were self-compatible and able to be cross-pollinated. Both artificial pollination tests on *T. laevis* had a significantly lower amount of seed set per fruit than the control. This phenomenon occurred due to inefficiency in hand pollen deposition. The stigma of *Tacca*'s flower was underneath the stigmatic lobes which made artificial pollination difficult to achieve. It was not possible to confirm the amount of pollen successfully placed on stigma. More samples should be tested if the availability of inflorescence is sufficient.

Controlled pollination experiment of *T. reducta* showed that it was able to perform both selfing and crossing. However, during experiments, most of the samples (21 out of 24) for artificial cross-pollination did not produce fruits. This amount was more than artificial self-pollination in which only seven out of 16 inflorescences did not produce fruits. Thus, this species was easier to undergo fertilization with selfing. These experiments showed that the mean values of seed set for control and artificial pollination tests were more or less equal. By assuming hand pollination was not efficient, the seed set in artificial pollination would be low. Thus, the fruits from open pollination also had low seed number. However, the main cause of this low seed set was not clear. The reason maybe infrequent visitation of pollinators, low resource allocation, or other environmental factors such as precipitation which may reduce the pollen viability.

Lastly, the inflorescences of all species did not fruit when they were isolated from any pollination chances. As suggested by Zhang et al. (2005), *T. chantrieri* adopted autonomous selfing mechanisms which allowed this species to reproduce without external aids. These

mechanisms included “close proximity of anthers and stigmas within flowers, anther dehiscence prior to flower opening, and additional self-pollination when flower stalks bend downwards on the second day of anthesis (delayed self-pollination)” (Zhang et al., 2005, p. 522). *Tacca* spp. in Sarawak had anthers dehisce after the flowers opened for one to two hours. This characteristic may be the reason *Tacca* spp. in this study didn’t produce fruit in autonomous self-pollination test. Moreover, high visitation rate of midges, which is absent in *T. chantrieri*, may ensure efficient cross-pollination, and thus reducing the selection towards self-pollination.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

In this study, the phylogenetic relationship of *Tacca* spp. was updated with species from Borneo, namely *T. bibracteata*, *T. borneensis*, *T. laevis*, and *T. reducta*. The data analysis of the combined dataset of nuclear ITS and plastid matK sequences showed that *T. bibracteata* formed a closer relationship with *T. palmata*, *T. palmatifida* and *T. plantaginea*. Other *Tacca* spp. endemic to Borneo formed a clade together with other species from Sumatra and Peninsular Malaysia. Within this clade, *T. borneensis* sister to the subclade of *T. cristata* and *T. laevis* and subclade of *T. reducta* and *T. sumatrana*. Relationship of other species was identical to the previous phylogeny study (Zhang et al., 2011).

According to morphological reconstruction, the ancestral traits of *Tacca* spp. were probably palmate leaf shape, green floral structures, long peduncle, big outer involucral bracts with numerous reduced inner involucral bracts, many long bracteoles, abundant of flowers per inflorescence, and a small number of ovules per flower. These characteristics indicated a conflict with the previous study on the evolution of floral traits of *Tacca* spp. which stated the changes from simple to elaborate floral characters (Zhang et al., 2011). In the future analysis, *T. ankaranensis*, *T. celebica*, *T. ebeltajae* and other species should be added to fully resolve the relationship within the genus. In the future study, the mating pattern can be investigated for each *Tacca* spp. in every population to evaluate the selfing rate based on molecular polymorphism. With this method, the general pollination strategy, either cross or self, used by different *Tacca* spp. can be predicted. Moreover, deducing the selfing rate can understand the efficiency of pollinator in cross-pollinating flowers of *Tacca* spp.

The flowering pattern of *Tacca* spp. in West Sarawak can be divided into three types. First, annual flowering with abundant of flowers; second, infrequent flowering with abundant of flowers; third, frequent flowering with few numbers of flowers. Floral anthesis of *Tacca* spp. was similar with middle-most buds initially bloomed, and floral movement included perianths opening and flower declinate. *Tacca leontopetaloides* anthesis period began in late evening, which was different from other species that bloom in the morning. Moreover, this species and *T. laevis* were detected to emit faint scent during anthesis. *Tacca leontopetaloides* and *T. palmata* had their perianth closed at the end of anthesis, covering the reproductive parts. The difference in floral phenology of observed *Tacca* spp. with *T. chantrieri* (Zhang et al., 2005) was the time of pollen extrusion in which all observed *Tacca* spp. had anthers dehiscence few hours after flower opening. Besides the differences in floral phenology, *Tacca* spp. also developed different infructescence type which was probably related to their seed dispersal strategies.

For floral characteristics, all species had a differentiable set of quantitative floral characters. The bracteoles number of *Tacca* spp. did not correspond with the number of flowers. The variation in ovules number and seeds number indicated that *Tacca* species develop the bet-hedging strategy by increasing ovule production to deal with stochastic pollination opportunity (Burd, 1995). In addition, all *Tacca* spp. had P:O ratio similar to facultative autogamy species (Cruden, 1977). This result suggested *Tacca* spp. probably established a mixed pollination system.

The pollinators of *Tacca* spp. were female Ceratopogonid midges. *Tacca borneensis* was visited by *Culicoides hinnoi*, and the rest of the observed species were visited by *Forcipomyia* sp. These insects were previously reported to visit *T. cristata* as well (Lim & Raguso, 2017). However, the strategy of *Tacca* spp. in attracting the pollinators was not

known. The behaviours of midges such as brood site preference and sexual response were important to understand the reason for their visits toward *Tacca* flowers which apparently did not offer any rewards for pollinators.

The absence of bracts and bracteoles in *T. borneensis* prevented the reproductive success, whereas in *T. reducta* both the fruit and seed set was significantly decreased. Although all fruit set of *T. cristata* and *T. laevis* was reduced, no significant difference was detected. Hence, the function of these conspicuous floral structures remained unclear in *T. cristata* and *T. laevis*.

For controlled pollination test, *T. laevis* and *T. reducta* were found to be self-compatible. Selfing in *T. borneensis* may result in early fruit abortion. Artificial pollination tests result of *T. laevis* showed significance in seed set against open-pollinated fruits. However, seed set depended on the amount of pollen deposited on stigma which was inconsistent due to the secured position of stigma within the flower. Thus, the seed set depended on the skill of hand pollination and the degree of mechanical damages caused during artificial pollination. Pollen supplementation test should be conducted to examine if heterogeneity of pollen deposition influences the seed set of *Tacca* spp. (Holland & Chamberlain, 2007). Moreover, these experiments should be performed under a more stable and constant environment, to prevent sample loss due to florivore or herbivore threats and weather damages. Other recommendation such as in vitro pollen viability test can be performed to test the germination of pollen in flower bud, anthetic flower and post-anthetic flower.

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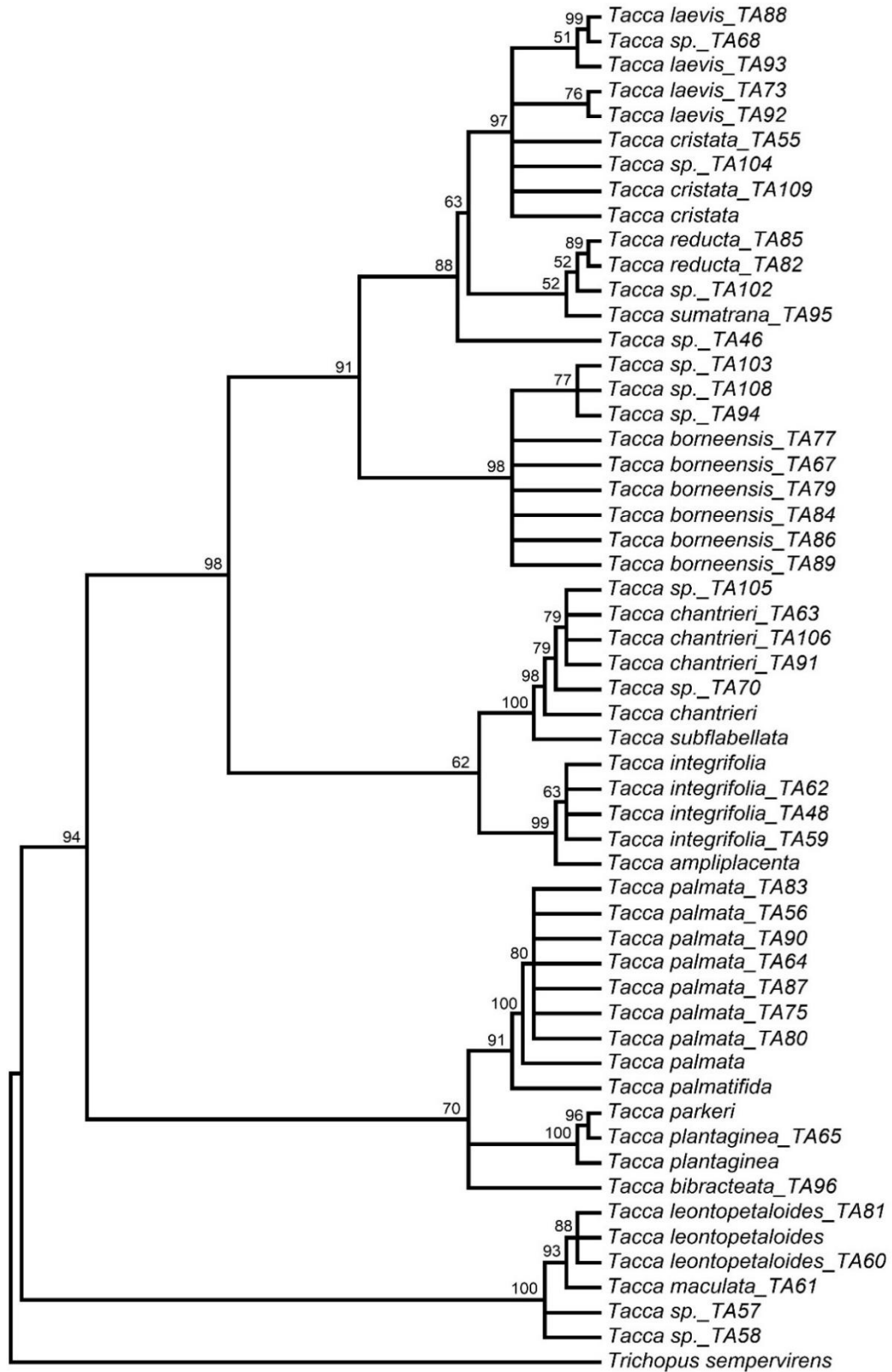
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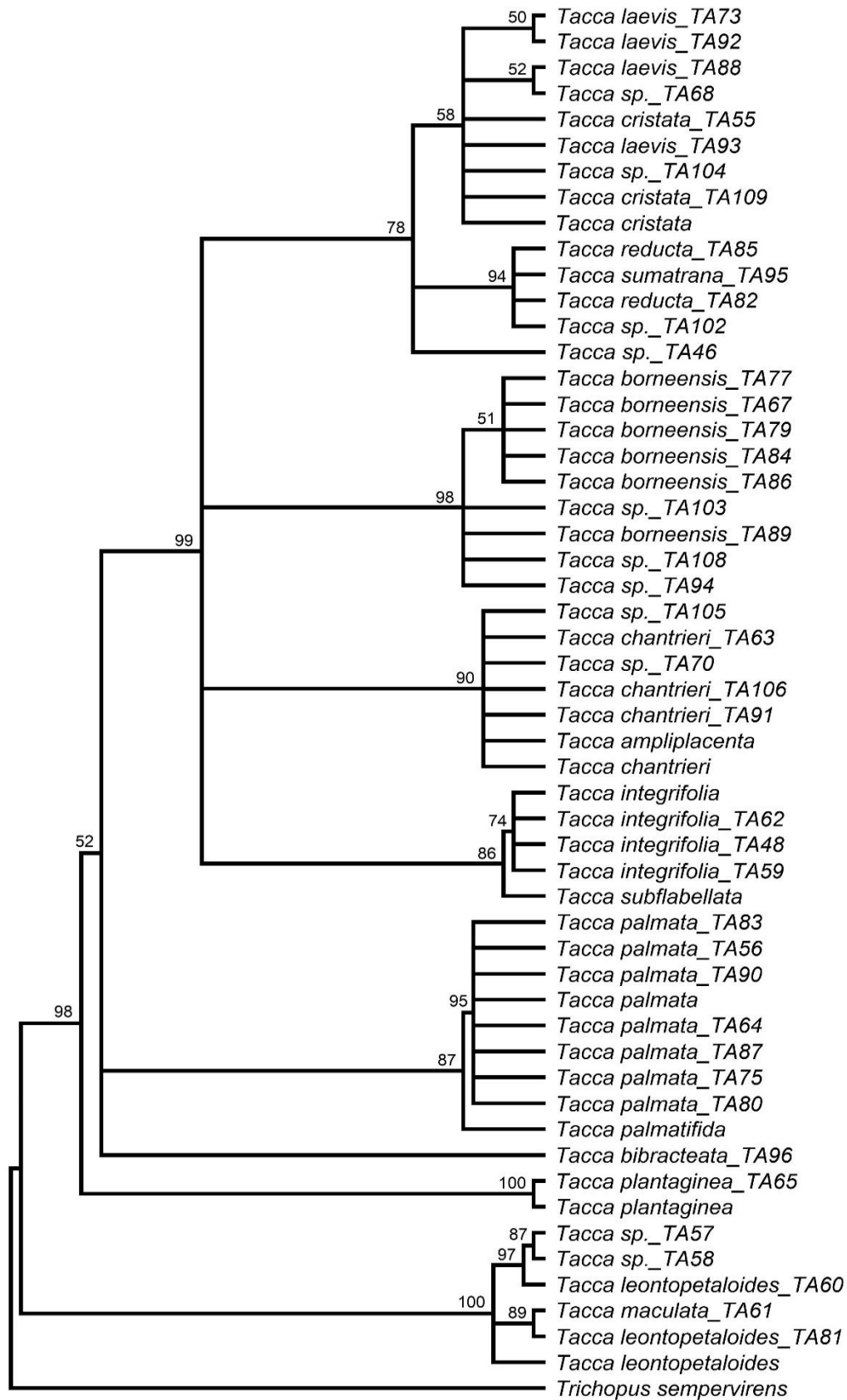
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APPENDICES

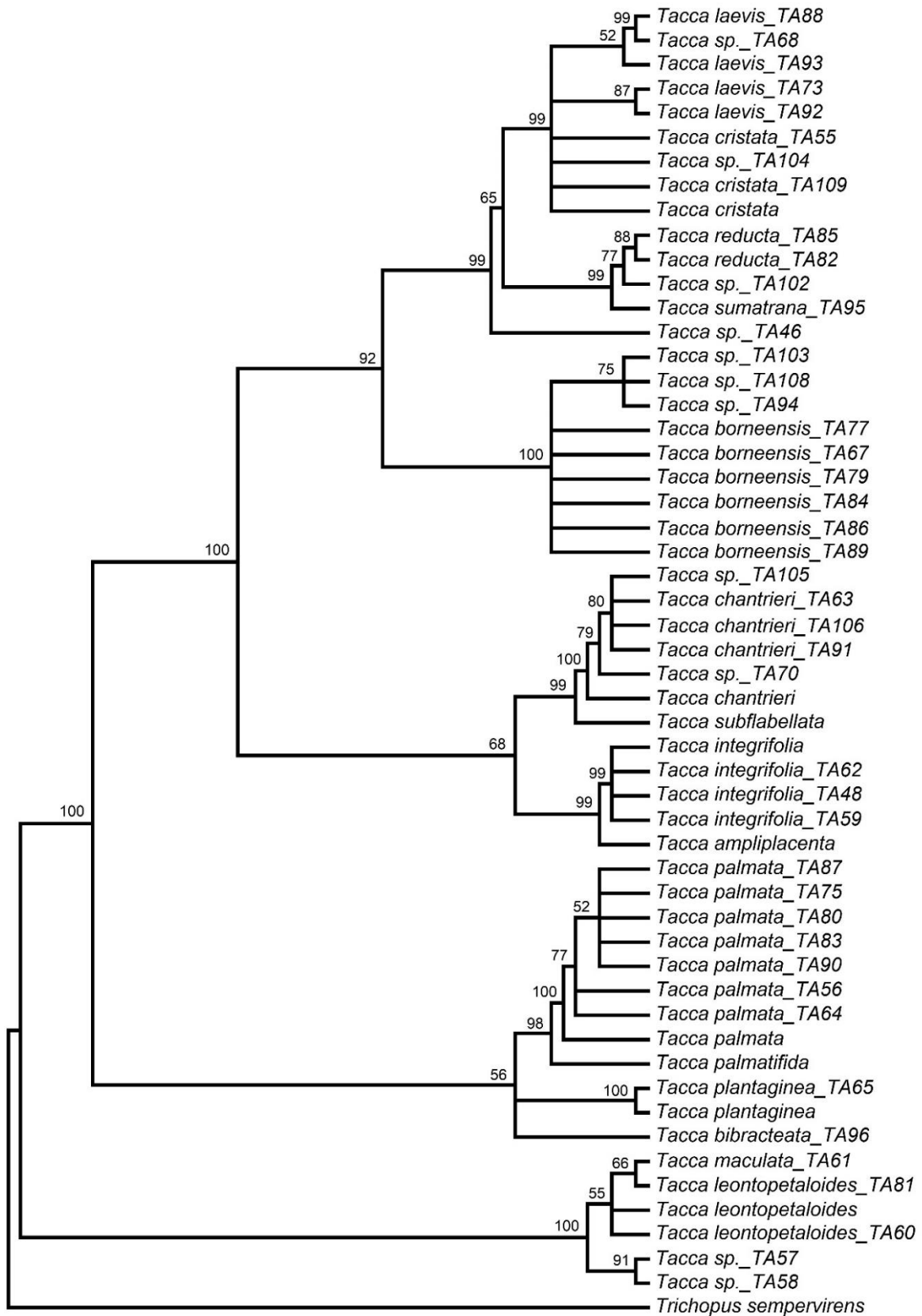
Appendix A1: MP analysis based on ITS region. Numbers above the branches are BS value.



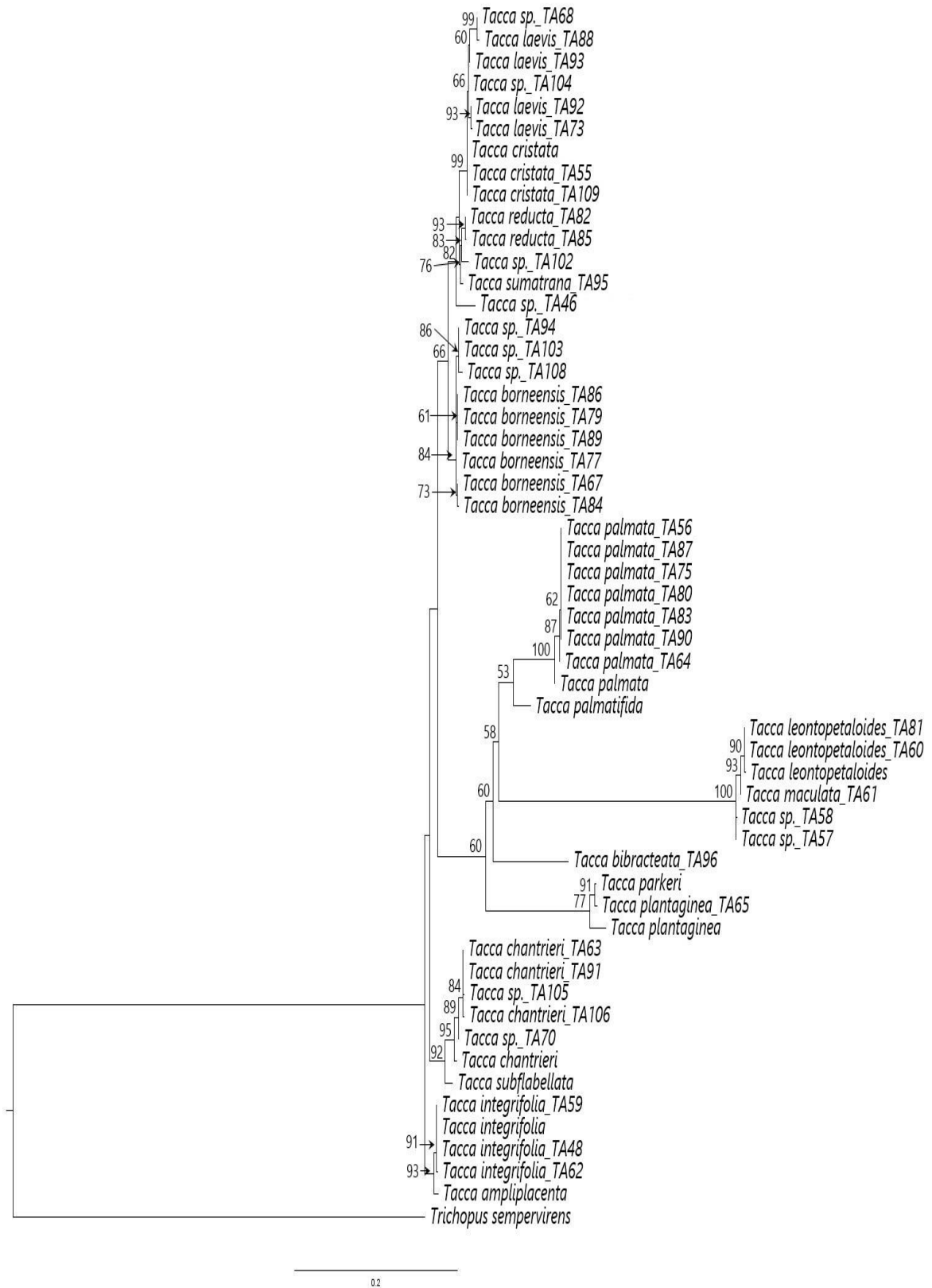
Appendix A2: MP analysis based on matK region. Numbers above the branches are BS value.



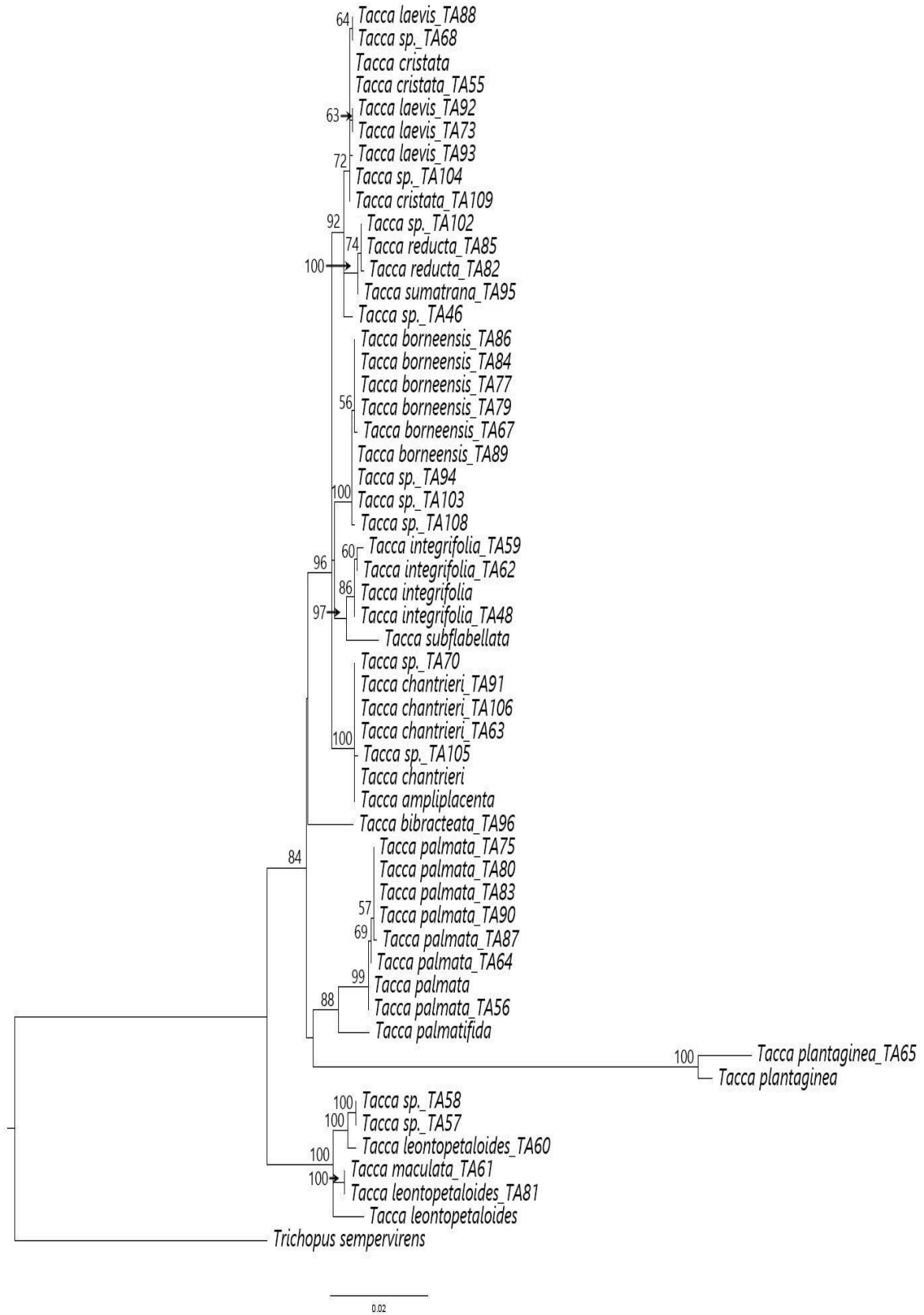
Appendix A3: MP analysis based on combined dataset. Numbers above the branches are BS value.



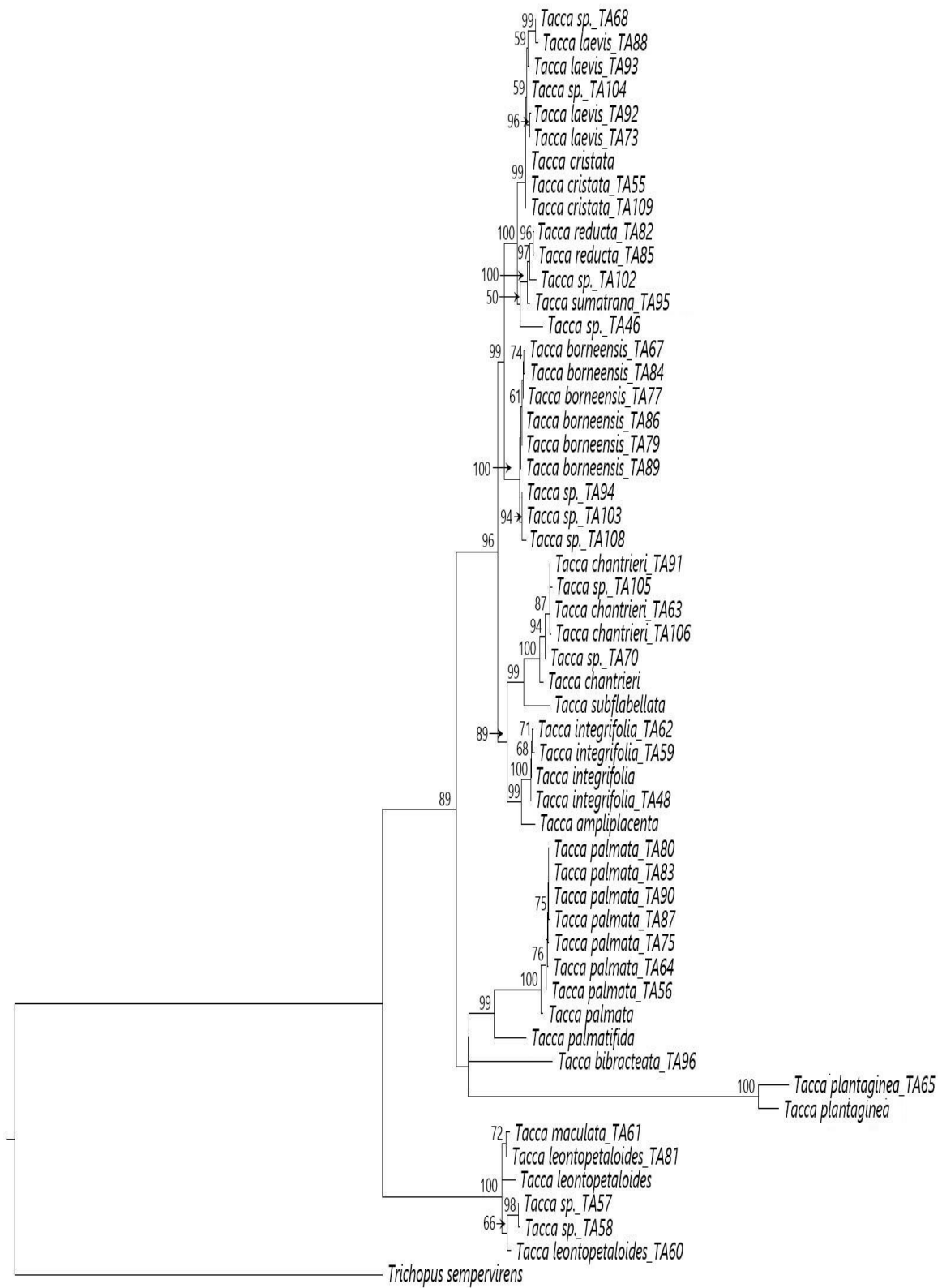
Appendix A4: ML analysis based on ITS region. Numbers above the branches are BS value



Appendix A5: ML analysis based on matK region. Numbers above the branches are BS value.

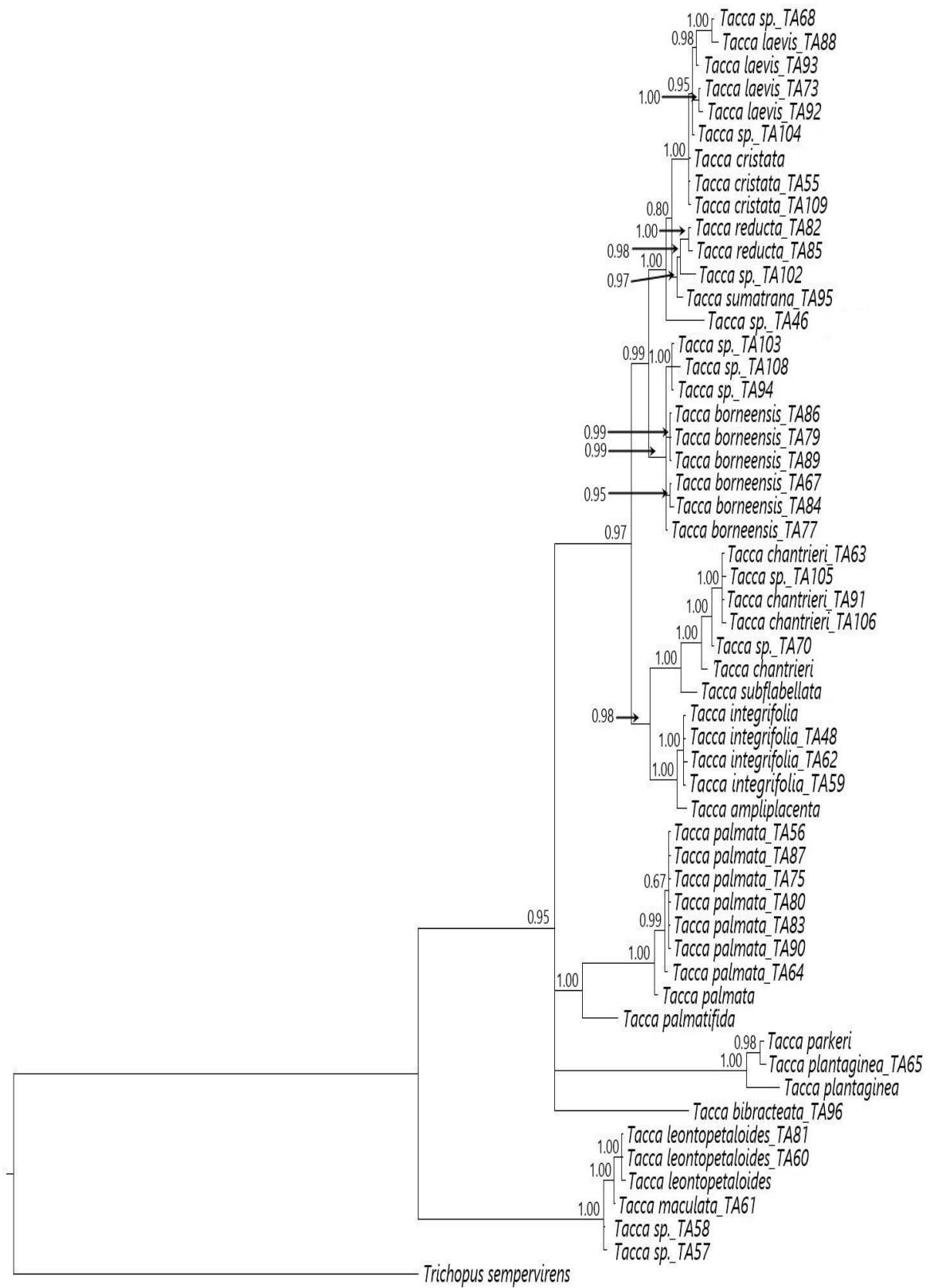


Appendix A6: ML analysis based on combined dataset. Numbers above the branches are BS value.

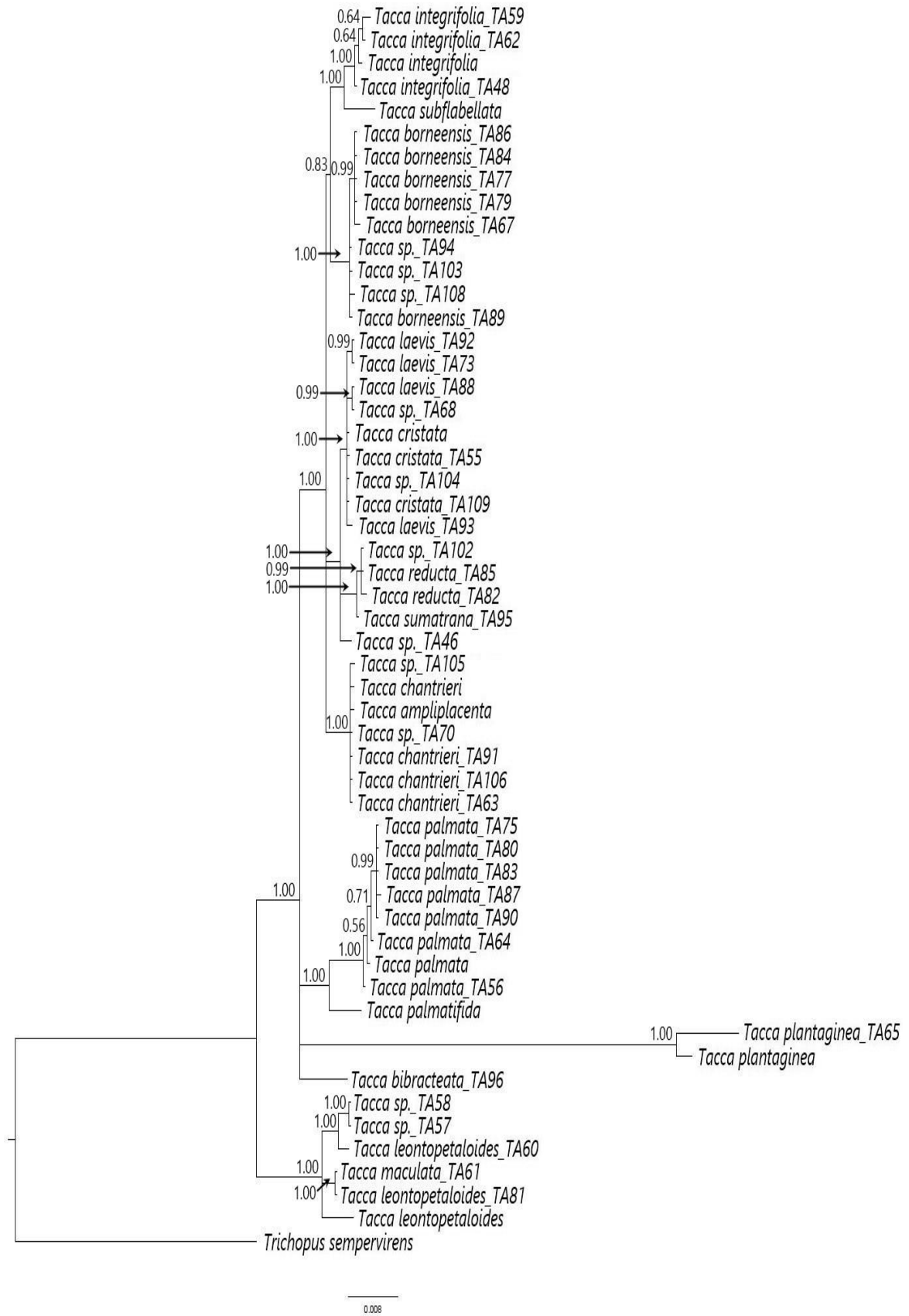


0.04

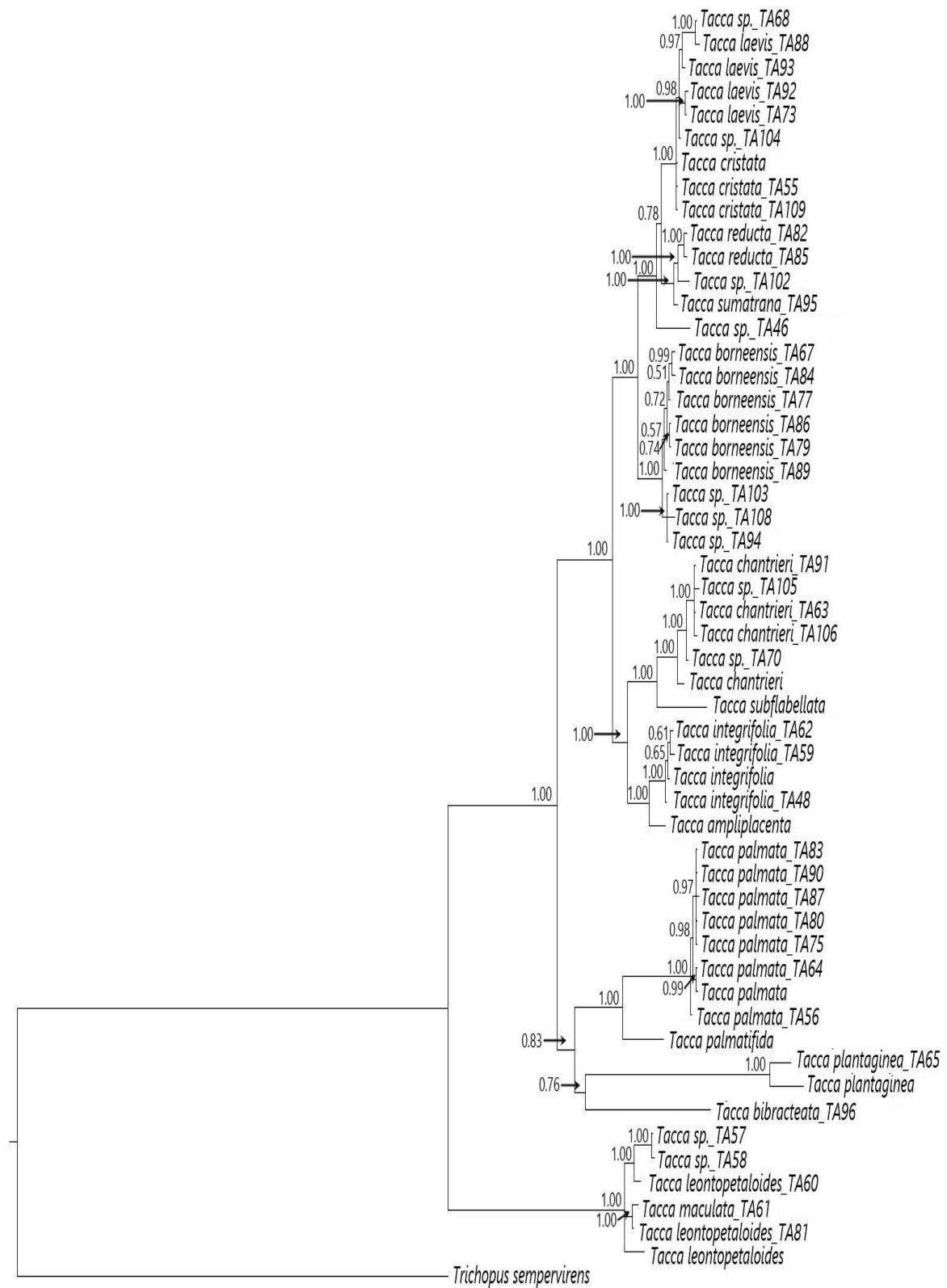
Appendix A7: BI analysis based on ITS region. Numbers above the branches are PP value.



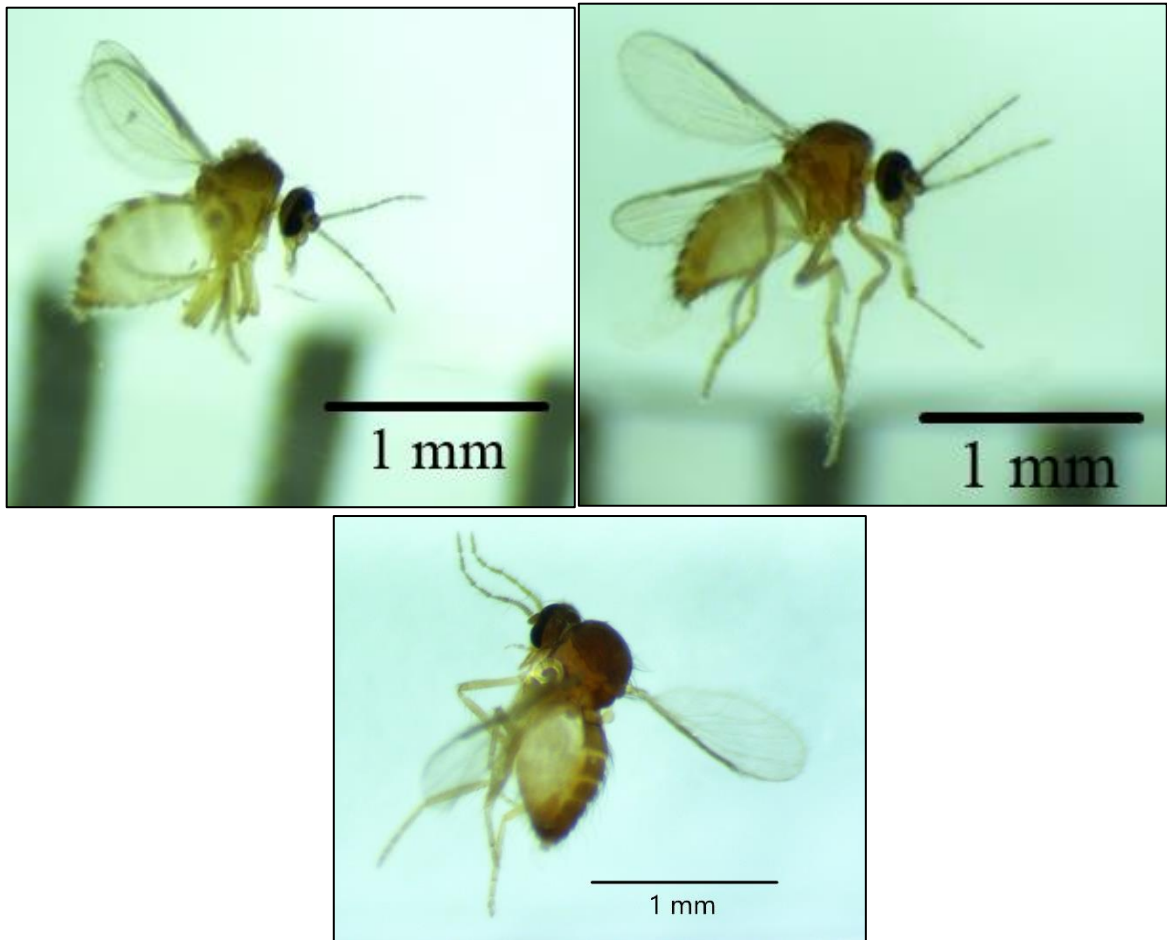
Appendix A8: BI analysis based on matK region. Numbers above the branches are PP value.



Appendix A9: BI analysis based on combined dataset. Numbers above the branches are PP value.



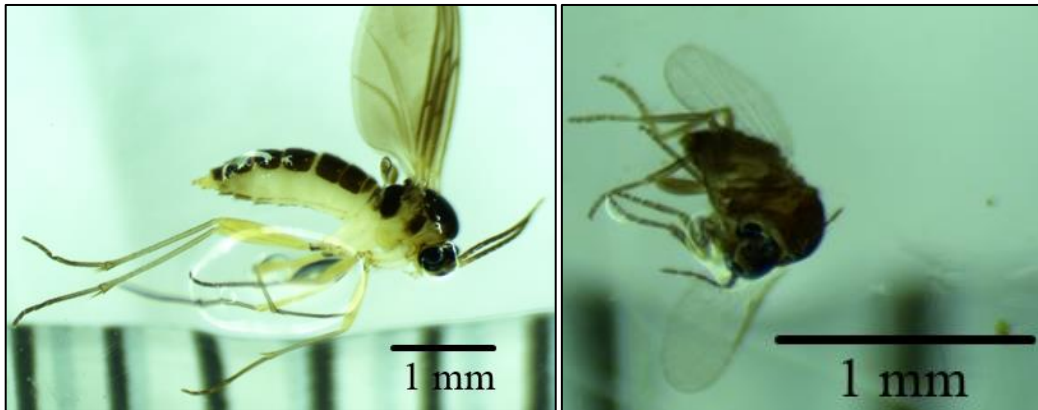
Appendix B1: *Forcipomyia* sp. 1 on *T. laevis* at KNP (left) and KS (right). *Forcipomyia* sp. 2 (bottom) on *T. laevis* at KS.



Appendix B2: *Forcipomyia* sp. 2 visited *T. reducta* (left) and *T. leontopetaloides* (right).



Appendix B3: Fungus gnat (Sciaridae; left) and *Forcipomyia* sp. 2 (right) on *T. cristata*.



Appendix B4: Female *Culicoides hinnoi* on *T. borneensis* at KNP.



Appendix B5: Fly (Acalyptratae; left) and *Forcipomyia* sp. (right) caught on *T. palmata* at RRP.

