Floral biology of *Schismatoglottis baangongensis* (Araceae) in West Sarawak, Borneo

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Abstract The flowering mechanism, visiting insect activities, reproductive system, and floral scent composition of *Schismatoglottis baangongensis*, a North West Bornean locally endemic limestone-restricted protogynous mesophyte were investigated. Anthesis started at dawn and lasted ca 29 hours. Fruit set for open pollination (93%) and restricted access pollination (88%) were high. *Colocasiomyia* (Diptera, Drosophilidae) and *Cycreon* (Coleoptera, Hydrophilidae) were the main pollinators. *Colocasiomyia* flies present in much higher numbers than *Cycreon* beetles individually carried significantly less pollen load. *Chaloenus* (Chrysomelidae, Galeuricinae) were inadvertent pollinators, and *Atheta* (Coleoptera, Staphylinidae) passive visitors. Pollen transferal between dissimilar insect genera (*Colocasiomyia* and *Chaloenus*) is reported for the first time. Low pollen-ovule ratio of *S. baangongensis* indicated an efficient pollination mechanism. Ester compound class floral odours, especially the dominant compounds 3-butenoic acid, 3-methyl-, methyl ester, were decisive in attracting pollinators. The spadix appendix of *S. baangongensis* was the main olfactory body although the spathe was detected to release an additional N-containing compound, an indole. An increase in the total amount of floral scent from the pistillate flower zone during pistillate phase of anthesis from Period I (0600 h–0800 h) to Period II (0800 h–1000 h) was postulated to detain insects in the lower chamber of the inflorescence.

Keywords Ester compound class, Floral volatiles, Pollen load, Pollination

Introduction

Schismatoglottis Zoll. & Moritzi is the largest genus in Tribe Schismatoglottideae, with about 120 described species out of an estimated 250 species (Boyce and Croat 2016; Hay and Yuzammi 2000; Boyce et al. 2010; Wong et al. 2010; Wong 2013). The type of the genus, *Schismatoglottis calyptrata* (Roxb.) Zoll. & Moritzi, is currently circumscribed as a polymorphic species occurring from central and eastern Indo-Malaya to northern Australasia (New Guinea and the Bismark Archipelago) as far east as Vanuatu (Hay and Yuzammi 2000; Wong 2012). Research in Sunda, however, is revealing a more intricate taxonomic situation with the existence of numerous locally endemic and geologically specialized species, the majority undescribed (Wong et al. unpublished data). Alongside a molecular analysis of species' relationships (Low et al. unpublished data) the opportunity to investigate pollination system was pursued: *Schismatoglottis baangongensis* S.Y.Wong, Y.C.Hoe & P.C.Boyce, a recently published species (Wong et al. in press) is the subject of this study.

Flowering biology and pollination investigations for Schismatoglottideae are limited to a few studies (Hotta et al. 1985; Sultana et al. 2006; Toda and Lakim 2011; Bröderbauer et

al. 2012; Wong and Boyce 2014; Low et al. 2014, 2016). Overall it appears that Schismatoglottideae species have a ca 30 hours anthesis cycle, with a release of floral odour (reminiscent of sweet fruity smell) to attract the insect visitors (Bröderbauer et al. 2012; Low et al. 2014, 2016). Floral rewards include stigmatic secretions and pollen (Low et al. 2014, 2016), but in some species (i.e. *Aridarum nicolsonii* and *Phymatarum borneense*), alimentary sterile structures as well (Low et al. 2016).

Species of *Colocasiomyia* (Diptera, Drosophilidae) were determined as pollinators in several representative taxa of Schismatoglottideae: *A. nicolsonii, Bucephalandra bogneri, P. borneense, Schismatoglottis* spp. and, *Schottarum sarikeense* (Hotta et al. 1985; Wong and Boyce 2014; Low et al. 2014, 2016). *Colocasiomyia* flies utilizing inflorescences for breeding and as brood sites were reported by Sultana et al. (2006) and Toda and Lakim (2011) for *Schismatoglottis corneri* (as "*Schismatoglottis* sp. H"), and for a new species of *Schismatoglottis* (reported as *S. calyptrata*), *Ooia kinabaluensis* (reported as *Piptospatha kinabaluensis*) and *Ooia grabowskii* (stated as *Piptospatha grabowskii* but misidentified as *Ooia kinabaluensis*; no other *Ooia* species occur at their study site). *Chaloenus* (Coleoptera, Chrysomeloideae) is known to pollinate *Bucephalandra aurantiitheca* (Wong and Boyce 2014). Field pollination studies in Araceae mostly recorded staphylinid beetles as insect visitors (García-Robledo et al. 2004, 2005; Tung et al. 2010; Chartier et al. 2011; Hoe et al. 2011, 2016; Takano et al. 2012; Low et al. 2014, 2016) or florivores (Gibernau et al. 1999).

Floral volatiles play an important role in attracting pollinators (Knudsen and Gershenzon 2006; Knudsen et al. 2006) functioning as a signal (Pellmyr and Thien 1986) to attract or guide pollinators to approach, land, seek reward (including sometimes mating & reproducing on the inflorescences), and to facilitate pollen transfer (Raguso 2001). However, the same volatiles that attract beneficial visitors can also attract visitors that have a negative effect on plant fitness, including herbivores, nectar robbers, and inefficient pollinators (Miyake and Yahara 1998, 1999). In Araceae, a temporal restricted scent emission is known to occur in *Alocasia* (Ivancic et al. 2005), *Homalomena* (Kumano and Yamaoka 2006, Kumano-Nomura and Yamaoka 2009; Hoe et al. 2016) and *Philodendron* (Gibernau et al. 1999; Pereira et al. 2014).

Field studies integrating pollination investigations with an assessment of the floral scent composition in Araceae are few, limited to temperate European *Arum* (Chartier et al. 2013, 2016; Kite 1995; Kite et al. 1998; Diaz and Kite 2002; Quilichini et al. 2010; Urru et al. 2010), Neotropical *Anthurium* (Hentrich et al. 2007, 2010; Schwerdtfeger et al. 2002), Neotropical *Caladium* (Maia et al. 2012, 2013b), temperate N American *Peltandra* (Patt et al. 1995), Neotropical *Philodendron* (Maia et al. 2010; Dötterl et al. 2012; Gottsberger et al. 2013), Neotropical *Spathiphyllum* (Hentrich et al. 2010), and Neotropical *Taccarum* (Maia et al. 2013b). Studies of tropical Asian taxa are limited to Miyake and Yafuso (2005) for *Alocasia*, and Kumano and Yamaoka (2006), Kumano-Nomura and Yamaoka (2009), Tung et al. (2010), Hoe et al. (2016) for *Homalomena*. In short, anthecology for tropical aroids remains largely unknown. This study aimed to investigate the pollination biology of *S. baangongensis* as part of an extensive survey of the taxonomically intricate *Schismatoglottis* Calyptrata Complex. This study was set out to specifically investigate: (1) the flowering mechanisms and pollination strategies, (2) the floral scent compounds, (3) the pollinator activities in relation to floral scent emission during the pistillate and staminate phases of anthesis, and (4) reproductive success of *S. baangongensis*.

Materials and methods

Study area and plant species

The study was conducted at the type locality of *S. baangongensis*, Baan Gong waterfall stream (01° 20' 16.1"; 110° 20' 09.6"), Kuching Division, Sarawak, Malaysia, NW Borneo. The study area is a lowland moist to wet evergreen tropical forest on karst limestone, 70–75 m above sea level, with an annual mean rainfall of 4,624 mm, annual mean temperature of 26.5ºC, and annual mean relative humidity of 85.5 % in 2011 (Sarawak Division, The Meteorology Department Malaysia). The study was carried out between December 2009 and December 2015. Specific dates for each experiment are provided in Table 1.

Schismatoglottis baangongensis is an evergreen colonial mesophyte occurring in patches of between 50–150 plants in shaded areas along forested streams on karst limestone. Shoots are hapaxanthic, with long-petiolate cordate-sagittate leaves, and terminating in a flowering event of up to five inflorescences. Each inflorescence is composed of a central flower-bearing spike (the spadix), subtended and enclosed by a modified bract (the spathe). The spathe is divided into a lower spathe and an upper spathe limb separated by a pronounced constriction coinciding with the sterile interstice of the spadix. The spadix is divided into four zones from the base: a pistillate zone, a sterile interstice, a staminate zone, and an appendix composed of staminodes. Pistillate flowers are irregularly accompanied by clavate interpistillar staminodes. Each ovary contains 17–53 ovules. Staminate flowers are mostly formed by two stamens, rarely three to four stamens. Inflorescences are protogynous and produce a strong esteric odour (Wong et al. in press).

Floral biology and floral visitors

Inflorescences (N = 5 from five individuals) were identified and observed for one week prior to the onset of anthesis. When anthesis was imminent, hourly observations were made until the end of anthesis. Anthesis stages were each found to be associated with distinctive activities in the inflorescence, respectively: gaping of the spathe limb, production of a stigmatic droplet, and start of odour production (onset of pistillate anthesis); tightening of lower spathe/spathe limb constriction, stigmas drying, and cessation of floral odour (end of pistillate anthesis/interfloral phase); expansion of spathe limb and pollen release (onset of staminate anthesis), and spathe limb degrading at its junction with the lower spathe, and shedding (end of anthesis). Anthesis phases, spathe movements, floral scent emission, pollen extrusion, and insect visitors were recorded. Behaviours of insect visitors, notably whether they made contact with stamens and stigmas were noted.

Twenty-three additional inflorescences from different individuals were bagged during pistillate anthesis to acquire data of total insect visitor numbers/per inflorescence, the visitation frequencies (percentage of visited inflorescences among the 23 observed inflorescences), and to enable identification of the insects. Insects were identified to the lowest possible taxonomic level (and at least to family) by M. J. Toda [Hokkaido University: drosophilid flies (*Colocasiomyia*)], H. Takizawa [Tokyo University of Agriculture: chrysomelid beetles (*Chaloenus*)], M. Munetoshi [Kyushu University Museum: staphylinid beetles (*Atheta*)], A. G. Kirejtshuk [Russian Academy of Sciences] and M. Fikáček [National Museum in Prague: hydrophilid beetles (*Cycreon*)]. All inflorescences, infructescences and insect visitors were preserved in 70% ethanol and deposited at the Sarawak Forestry Herbarium (SAR) and Sarawak Forestry Entomology Museum.

To confirm the presence of pollen on the bodies of the suspected pollinators, insects were collected upon arriving at inflorescences and dried in silica gel for three days. Pollen was obtained from the host plants as well. The pollen samples were mounted on metal stubs

and sputter-coated with gold. Specimens were viewed under a JEOL JSM-6390LA scanning electron microscope (SEM) with a digital imaging capacity at 10kV.

Reproductive system

Four pollination treatments (open pollination, restricted access pollination, cross pollination, and autonomous self-pollination) were applied to *S. baangongensis*. Natural pollination and fruit set was assessed from unbagged inflorescences open to natural pollination (Treatment 1: open pollination, N = 26). To confirm that *Colocasiomyia* flies and *Cycreon* beetles pollinated *S. baangongensis* (see results), a restricted access experiment (restrict larger *Chaloenus* beetles and *Parastasia* beetles) was undertaken by using inflorescenses about to open and enclosed in 2 mm x 2 mm mesh bags (Treatment 2: restricted access pollination, N = 18). The mesh bags were removed at the end of the anthesis. In the treatment of cross pollination, inflorescences about to open were bagged from 0400 h onwards to exclude all floral visitors. Staminate flowers were emasculated immediately at the onset pistillate anthesis and receptive pistils (between 0800 h and 0830 h) brushed with pollen collected between 0400 h–0530 h on the same day from nearby plants, with floral visitors excluded by enclosing the pollinated inflorescences in organdy bags until the end of anthesis (Treatment 3: cross pollination, N = 8). Autonomous self-pollination and fruit set was tested using inflorescences about to open which were bagged to exclude all floral visitors (Treatment 4: autonomous self-pollination, N = 5).

Fruit set percentage was used to measure pollinators effectiveness, and was defined as the ratio between the average of the total number of fruits per infructescence and the average of the total number of pistillate flowers per inflorescence. Fruits resulting from the experimental inflorescences were collected and counted after one month. Seed set was estimated by counting the number of seeds in 10 developed fruits selected randomly from ten infructescences (eight infructescences in Treatment 3).

Pollen-ovule ratio (P/O) was calculated from the number of ovules and the number of pollen counted from 10 inflorescences using an Olympus BX51 compound microscope. The total number of ovules per inflorescence was determined by counting the mean number of ovules of 10 pistillate flowers, multiplying by the mean total number of pistillate flowers. Pollen counts were done using three groups of 10 stamens prepared by digestion for five days at room temperature (25.5–27.5 ºC) in 600 µl of 95 % sulphuric acid. The resulting solution was homogenized, and the number of pollen from one µl was counted using a Sedgewick Rafter Counting Chamber (S52-glass) for three independent replicates. The total number of pollen grains per inflorescence was obtained by multiplying the mean of three independent replicates by 600, dividing by 10, and multiplying by the mean total number of stamens.

Pollen loads

Pollen loads of four types of insects (per individual) were quantified to determine its efficiency as pollinators. Inflorescences were bagged with mesh bags (0.5 mm x 0.5 mm mesh) prior to anthesis (N = 13, different individuals). During pistillate anthesis (0600 h–0900 h), insects were individually captured upon arrival (*Colocasiomyia* flies, N = 58; *Cycreon* beetles, N = 52; *Chaloenus* beetles, N = 6; *Atheta* beetles, N = 4). Insects were individually stored in 2 mL collecting tubes and preserved at -20 °C until further analysis. Individual insects were immersed in 200 µl of 70 % ethanol to remove the pollen from the body. Forty µl of the post-immersion solution was transferred onto a Sedgewick Rafter

Counting Chamber (S52-glass) and left covered until the alcohol had evaporated. Pollen counting was done using an Olympus BX51 compound microscope equipped with the Olympus DP72 camera and the Cell ^D software, version 3.3 (Build 2067).

Sampling and analyses of floral scent

Floral volatile organic compounds (VOCs) of *S. baangongensis* were sampled in its natural population using the dynamic headspace method following Raguso and Pellmyr (1998). VOCs were trapped for two hours between 0530 h–0800 h for pistillate phase (N = 6, different plant individuals). VOCs were also trapped for two hours between 0530 h–0800 h for staminate anthesis (N = 2) to confirm that floral scents, then imperceptible to the human nose, were not emitted. In order to ascertain the physical origin of VOCs, an additional set of spadices was divided into constituent floral structures in the natural population of *S. baangongensis*. The VOCs were trapped twice: Period I: pistillate phase of anthesis between 0600 h–0800 h (appendix, N = 2, pistillate zone, N = 4, spathe, N = 3, and staminate zone, N = 3), and Period II: pistillate phase of anthesis between 0815 h–1015 h (appendix, N = 2, pistillate zone, N = 2, spathe, N = 2, and staminate zone, N = 4).

VOC sample was obtained by enclosing individual inflorescence in PET film oven bags (EasyRoast™, Bacofoil, UK), from which the air was drawn by a battery-operated vacuum pump at a constant flow rate of 200 ml per min (Spectrex PAS-500 Micro Air Sampler; Spectrex, USA). A clean needle (1 mm diam.) was used to make ca 10 perforations in the oven bag to enable airflow through the bag. VOCs were trapped in a glass tube containing 150 mg of an adsorbent polymer (ORBO™ 402 Tenax® 35–60 mesh, Sigma-Aldrich, USA). A control per sample was collected simultaneously using an empty oven bag. When sampling was not being carried out, inflorescence was covered with a fine organdy bag to prevent insect visitation. The adsorbent traps were eluted with hexane (4 mL; ≥ 98.5% purity; MERCK, Germany), and stored at -20 °C until analysis.

Prior to analyses, 5 µL of tetradecane (internal standard, 13 ng/µL) was mixed with 600 µL of the hexane eluate (see above). Each sample was then concentrated to 80 µL under a fume hood. Analyses were conducted by combined gas chromatography-mass spectrometry (GC-MS) on a Shimadzu GCMS-QP2010 Plus (Shimadzu Corporation, Kyoto, Japan), equipped with a BP20 polar column (SGE Analytical Science; 30 m long, 0.25 mm inner diameter and film thickness 0.25 um). For each concentrated sample, 1 µL was injected in split mode (1:20) with the injector temperature set to 200 °C. GC oven temperature was set at 50 °C for 5 min, then increased at a rate of 5 °C min^{-1} to 250 °C, then held steady for 10 min. The carrier gas flow was maintained at a constant pressure of 100 psi. MS Source and quadrupole temperatures were set at 220 °C and 200 °C, respectively. Mass spectra were taken from *m/z* 35–500 in EI mode.

Kovats Retention Indexes (KIs) of the VOCs were obtained with an external standard (C_8–C_{22} saturated alkanes, Supelco, USA). Compounds were tentatively identified by cross-referencing their mass spectra and retention times with data from commercially available mass spectral libraries (nist08 Library Mass Spectral Database). The peak areas on the chromatograms were manually integrated to obtain the total ion current signal, which was used to determine the relative amount of each compound.

Statistical analyses

Throughout the study, Kolmogorov-Smirnov tests were performed on the obtained datasets. All datasets were not normally distributed, and thus non-parametric Kruskal-Wallis tests were applied. For number of insects and number of pollen, a log-transformation was applied to the dataset before the non-parametric Kruskal-Wallis test was applied. Post-hoc Mann-Whitney tests were applied on each pair of groups taking into account the Bonferroni correction. All statistical tests were carried out using Paleontological Statistics (PAST) version 2.17 (Hammer et al. 2001).

Results

Floral biology

Anthesis in *S. baangongensis* lasted 29 hours (Fig. 1). A day prior to opening, the inflorescence turned paler green (Fig. 2a). The pistillate phase of anthesis started at 0400 h, when the spathe inflated (Fig. 2b), a gap was formed along the spathe limb and the lower spathe margin, and stigmatic droplets appeared. Concomitantly, a mild esteric scent was detected. By 0600 h, the spathe limb opened wide (ca 6.5 cm long x ca 3.3 cm wide; Fig. 2c) and the lower spathe inflated (ca 4 cm long x ca 2.3 cm wide). Between 0720 h–0930 h, the spathe limb (now ca 3 cm wide) and the lower spathe (now ca 2 cm wide) tightened back. Floral scent production ended by 1000 h and stigmas dried up. Staminate phase of anthesis started on the following day by the abscission of the spathe limb from 0500 h (Fig. 2d) and the release of powdery pollen acroscopically between 0510 h–0530 h. After 35–40 days, the persistent lower spathe split medioscopically or basiscopically to expose the ripe fruits. The fruits and seeds were observed to be dispersed by unidentified black and red ants (Online Resource 1).

Floral odour

Four VOCs belonging to the ester compound class were identified from *S. baangongensis* during pistillate phase of anthesis (Table 2). High relative amounts of 3-butenoic acid, 3-methyl-, methyl ester, and low relative amounts of 2-butenoic acid, 3-methyl-, methyl ester, 3-buten-1-ol, 3-methyl-, and methyl benzoate were detected. Kruskal-Wallis test ($p > 0.05$) indicated scent profiles among the replicates were similar. No VOCs were detected during staminate phase of anthesis.

Five VOCs were identified from the isolated inflorescence structures (spathe, pistillate flower zone, staminate flower zone, appendix) for Period I and II, with, additional to the VOCs detected during field sampling, one VOC in the nitrogen compound class (indole). At both time periods, the appendix and spathe released the highest number of VOCs (4–5 VOCs), followed by the staminate and pistillate zone (two VOCs). All inflorescence structures emitted a high relative amount of 3-butenoic acid, 3-methyl-, methyl ester and small to moderate relative amount of methyl benzoate at both periods. The appendix and the spathe emitted only small relative amounts of 2-butenoic acid, 3-methyl-, methyl ester, and 3-buten-1-ol, 3-methyl-. Only the spathe emitted indole at both periods (Table 2).

At Period I, the appendix emitted the highest total amount of floral VOCs, following by the spathe, the staminate zone and the pistillate zone (Table 2). At Period II, the total amount (ng/hr) of floral scent was the highest in appendix, followed by the staminate zone, the pistillate zone, and the spathe. The total amount of VOCs of the spathe, the staminate zone and the appendix were reduced by $^{1}/_{4}$–$^{4}/_{5}$ between Period I and Period II. However, the

total amount of VOCs increased almost twice in the pistillate zone. Kruskal-Wallis test showed no significant differences ($p > 0.05$) for the total amount of VOCs among the inflorescence structures.

Reproductive system

Infructescences from open pollination had the highest fruit set (93 %, Table 3) while restricted access pollination was lower, but not significantly at 88 % ($p > 0.05$). Infructescences resulting from hand-crossing experiments (xenogamy) had a significantly lower fruit set at 36 % ($p < 0.05$, as compared to open pollination and restricted access pollination). Inflorescences subjected to autonomous self-pollination treatment set no fruits. Thus *Schismatoglottis baangongensis* is wholly reliant on pollinator visits for fertilization and seed production. Seed set was not significantly different ($p > 0.05$) among the successful pollination treatments (open pollination, 12 ± 3; restricted access pollination, 11 ± 1; and cross pollination, 8 ± 21).

The number of pollen grains per stamen varied from 9,300 to 36,120 (average 16,337 ± 4,052). Consequently, the number of pollen grains per inflorescence was highly variable, ranging between 13,419,900–52,121,160 (average 23,574,002 ± 5,846,503). The number of ovules per pistillate flower varied from 17–53 (average 27 ± 7), yielding the number of ovules per inflorescence a range of 13,566–42,294 (average 21,402 ± 5,521). At individual flower level, the P/O ratio was 637 ± 192. At the inflorescence level, the P/O ratio was 1,152 ± 347. The difference between the values of P/O ratios for the inflorescence and the flower, is owing to the ratio between staminate and pistillate flowers in the inflorescence. The greater the difference between the number of staminate and pistillate flowers is, the greater the difference between the flower P/O ratio and inflorescence P/O ratio.

Floral visitors

Colocasiomyia flies and *Cycreon* beetles arrived at the inflorescences of *Schismatoglottis baangongensis* during pistillate anthesis between 0620 h–0720 h. *Colocasiomyia* flies were highly active, moving freely within the inflorescences. The flies were observed to feed on the stigmatic fluid secreted during the pistillate phase, exudations from the interpistillar staminodes (Fig. 2f), and appendix tissues previously damaged by *Chaloenus* beetles. Mating (inner spathe, pistillate zone, staminate zone, and appendix) and ovipositing (pistillate zone, staminate zone, and appendix) activities of *Colocasiomyia* flies were observed. *Cycreon* beetles mostly remained within the lower spathe chamber and were observed to consume secretions on the interpistillar staminodes (Fig. 2f), and to mate on the pistillate zone.

Between 0720h–0930h, *Chaloenus* beetles were observed to consume the interpistillar staminodes (Fig. 2g), and the appendix staminodes, and mate on the pistillate zone and the appendix. *Atheta* beetles were observed to move between the pistillate flowers (between 1530h–1600h). At the onset of staminate phase of anthesis, *Atheta* which remained on the inner side of the spathe limb left the inflorescences as the spathe limb abscised (Fig. 2d, white arrows) without making a contact with the staminate zone, and thus pollen load was not possible. As floral scent decreased, with the exception of *Chaloenus* beetles which remained on the appendix, all insects were less active and remained on the inside of the lower spathe chamber until the following day.

Pollen transfer

Colocasiomyia flies consumed pollen from the entire staminate zone while *Cycreon* beetles mostly consumed pollen from the lower part of the staminate zone. Pollen fully adhered on *Colocasiomyia* flies and *Cycreon* beetles (Fig. 2e). *Chaloenus* beetles remained on the appendix and laid eggs there (Fig. 2i). Thus direct pollen load was improbable although *Colocasiomyia* flies frequently crawled onto *Chaloenus* and thereby transferred some adhered pollen to the *Chaloenus* beetles (Fig. 2e, h). By 0615 h, *Colocasiomyia* flies and *Cycreon* beetles began to leave the inflorescences with pollen loads. *Chaloenus* beetles began to leave by 0635 h, and all types of insects departed the inflorescences by 0900 h. During collection for insect counts, three inflorescences (out of 23) were found with one to five individuals of *Parastasia* Westwood (Coleoptera, Scarabaeidae, Rutelinae) beetles.

The captured insect individuals per inflorescence were: *Atheta* beetles (4 ± 3), *Chaloenus* beetles (4 ± 4), *Colocasiomyia* flies (175 ± 108), *Cycreon* beetles (33 ± 31), and *Parastasia* beetles (2 ± 2) as shown in Table 4. The visitation frequencies of the insect genera were: *Atheta* beetles (47.8%), *Chaloenus* beetles (91.3%), *Colocasiomyia* flies (100%), *Cycreon* beetles (100%), and *Parastasia* beetles (13%) of the 23 sampled inflorescences. *Colocasiomyia* flies were identified as *C.* aff. *bogneri*; *Chaloenus* beetles as *C. dohertyi*, *C. latifrons*, *C. schawalleri* and *C.* sp. 1; *Parastasia* beetles as *P. gestroi* and *P. nigripennis* (Table 4).

Pollen load of *Cycreon* beetles was significantly higher than pollen load of *Colocasiomyia* flies and *Chaloenus* beetles (Table 5). *Atheta* beetles were not found to carry pollen. *Parastasia* beetle was not sampled for pollen load, since *Parastasia* visits were rare during the sampling period. Other types of unidentified pollen grains were also found on *Cycreon* beetles and *Colocasiomyia* flies (Online Resource 2).

Under SEM, pollen of *S. baangongensis* (Fig. 3a) matched pollen carried by *Cycreon* beetles and *Colocasiomyia* flies . However, pollen adhered onto different parts (abdomen, elytra, femur, pronotum, tarsi and tibia; Fig. 3c, d) of *Cycreon* beetles but only on the tarsi of *Colocasiomyia* flies (Fig. 3b). Thus, although the number of visiting *Colocasiomyia* flies was significantly higher than the number of *Cycreon* beetles, *Cycreon* carried significantly more pollen load. This indicated that with the timing and frequency of the *Cycreon* visits, *Cycreon* appears to be the most effective pollinators of *S. baangongensis*.

Discussion

Floral biology

Schismatoglottis baangongensis is an early morning flowering species, is similar to other observed species of Schismatoglottideae except *Schottarum* (Low et al. 2014, 2016). In common with aroids (except some *Anthurium*), pistillate phase of anthesis begins and ends before pollen release (Mayo et al. 1997; Chouteau et al. 2008). The spathe trapping mechanism of *S. baangongensis* is somewhat similar to the description of spathe trapping in Schismatoglottideae by Bröderbauer et al. (2012) in which “insects are trapped by temporary closure of the spathe blade”. However, Bröderbauer et al. (2012) made the observation based on *Apoballis*, which is an atypical representative of Schismatoglottideae with a persistent spathe limb till fruiting stage, spiny pollen and floral odour resembling almond oil. The spathe limb of *S. baangongensis* was observed to abscise at the onset of staminate anthesis. The spathe trapping mechanism of *S. baangongensis* is dissimilar to that of *Phymatarum borneensis* (Low et al. 2016) in which the spathe limb inflates during pistillate anthesis to

create a gap ca. 1 mm wide, permitting access to the pistillate zone only for small insects such as *Colocasiomyia* flies (Low et al. 2016), yet the wider space at the spathe constriction zone of *S. baangongensis* allow larger size insects (such as chrysomelid beetles) to have access into the pistillate zone.

The P/O ratio is limited to several studies in Araceae (Ramirez and Seres 1994; Chouteau et al. 2006a, 2006b, 2008; Gibernau et al. 2010; Maia et al. 2013a; Pereira et al. 2014). Several genera from old world tropics were studied (*Alocasia, Aridarum, Colocasia, Culcasia, Homalomena, Ooia* (reported as *Piptospatha grabowskii* previously; Wong and Boyce 2010), *Piptospatha, Pseudodracontium, Rhaphidophora, Schismatoglottis, Typhonium*) (Chouteau et al. 2008; Gibernau et al. 2010). Chouteau et al. (2006b) showed that facultative xenogamous species have higher P/O ratios than obligatory xenogamous species; this is congruent with the low P/O ratio in *S. baangongensis*, an obligate xenogamous species. The low P/O ratio at flower and inflorescence levels of *S. baangongensis* are within the range of reported P/O ratios of the inflorescences in Schismatoglottideae (Gibernau et al. 2010), and is also similar to *Philodendron* species, notably *P. melinonii* Brongn. ex Regel (Chouteau et al. 2006b). This implies a highly efficient pollination mechanism in Schismatoglottideae and *Philodendron* species, with complex pollinating mechanisms involving spathe movements, floral rewards, and the production of odour and heat (Gibernau et al. 1999, 2003; Seymour et al. 2003; Low et al. 2014, 2016).

Pollinators and other anthophilous insects

Floral scent is a possible attracting factor for five different insect taxa, with the families Drosophilidae and Hydrophilidae accounting for 81.9 % and 15.4% of the visitors respectively. Other insect visitors were Staphylinidae (0.9 %), Chrysomelidae (1.7 %), and Scarabaeidae (0.1 %). To be considered a legitimate pollinator of *S. baangongensis*, the visitor had to come into contact with the reproductive organs of the flower (pistillate staminate and flower zones) at the suitable time (pistillate phase of anthesis for the pistillate flowers, and staminate phase of anthesis for the staminate flowers).

The pollinators of *Schismatoglottis baangongensis* are *Colocasiomyia* flies, *Cycreon* beetles, and *Chaloenus* beetles. The first two are the primary pollinators as fruit sets were statistically similar between the treatments of open pollination and restricted access pollination. The pollen load counts for both insects were comparatively high. *Cycreon* beetles, however, carried more pollen than *Colocasiomyia* flies and may be the most effective pollinator. However, the number of *Colocasiomyia* individuals was ca six times higher than *Cycreon* beetles. *Cycreon* beetles are recorded here for the first time as pollinators for Araceae. In *S. baangongensis*, pollination is achieved by a diverse group of insects, each with differences in visitation frequency, timing and behaviour during visits, overall probably contributing to the high reproductive success. It is suggested that the odour in the floral chamber provides the source of attraction and the mating stimulus to pollinators.

Chaloenus beetles are considered as florivores and inadvertent pollinators. *Chaloenus* was reported as pollinators in Tribe Homalomeneae (Kumano and Yamaoka 2006; Hoe et al. 2011) although significantly damaging the spadix owing to the consumption of interpistillar and interstice staminodes, and the expanded connective tissue of the staminate flowers. As *Chaloenus* beetles mostly remained on the appendix, direct pollen adhering is rather improbable. However, *Colocasiomyia* flies frequently crawled onto *Chaloenus* beetles and thus, transferred some adhered pollen onto these beetles.

Atheta beetles visited the inflorescences of *S. baangongensis* as a food source (eggs of *Colocasiomyia* flies). This was reported in *Alocasia* as well (Takano et al. 2012). *Parastasia*

beetles are considered merely visitors since their occurrences were highly infrequent and high fruit set was observed with the exclusion of *Parastasia* beetles. The beetles' behaviour in the inflorescences was not observed, but nonetheless *Parastasia* beetles are unlikely to have a major role as pollinators owing to the absence of resin-mitigated pollen paste in *S. baangongensis*. Utilization of resin for pollen adherence is known for plant taxa pollinated by *Parastasia* beetles (Tung et al. 2010; Hoe et al. 2011, 2016) as this ensures pollen adherence (Gibernau and Barabé 2002). Utilisation of resin for pollen adherence has also been observed in associations involving plants of the Neotropical genus *Philodendron* and cyclocephaline scarabs (Scarabaeidae, Dynastinae; Mayo 1991; Maia et al. 2010; Gottsberger et al. 2013).

Floral odour

Analyses of floral scent samples showed that *S. baangongensis* emitted four types of ester compound class with a single major VOC, 3-butenoic acid, 3-methyl-, methyl (> 99.44 %). To date, 3-butenoic acid, 3-methyl-, methyl ester was only reported as a minor VOC compound in the petals of *Robinia pseudoacacia* (Fabaceae; Aronne et al. 2014). The scent of *S. baangongensis* is dominated by a typical esteric acid smell and thus it is not surprising that the VOCs comprise only compounds belonging to ester compound class. In Araceae, only appendix of *Sauromatum guttatum* was found to emit as high as 71% of ester compounds (Hadacek and Weber 2002). Skubatz et al. (1996) reported that the appendix of *Sauromatum venosum* attracts a variety of insects, including beetles, flies, wasps, and earwigs. A dominant compound, 3-butenoic acid, 3 methyl-, methyl ester, is reported here to occur in aroid species for the first time. A small relative amount of 2-butenoic acid, 3-methyl-, methyl ester (< 2.2 %) were reported from floral odours of *Cycas rumphii* (Cycadaceae) (Pellmyr et al. 1991), *Narcissus* spp. (Amaryllidaceae) (Dobson et al. 1997) and the petals of *R. pseudoacacia* (Aronne et al. 2014). A small relative amount of 3-buten-1-ol, 3-methyl- (< 2.7 %) was present in floral scents of *Narcissus* spp. (Dobson et al. 1997) and the sweet pea fruit of *Lathyrus odoratus* (Porter et al. 1999). Benzoic acid, methyl ester is commonly found in various angiosperms families as minor relative amount (< 5 %) in Araceae (Schwerdtfeger et al. 2002), Asteraceae, Caryophyllaceae, Polemoniaceae, Rubiaceae, Verbenaceae (Andersson et al. 2002) or as major relative amount (44.53–91.6 %) in Amaryllidaceae (Dobson et al. 1997), Caryophyllaceae (Jürgens, et al. 2002) and Nyctaginaceae (Levin et al. 2001). However, the roles of pollination attraction of these compounds were not further discussed except by Dobson et al. (1997) which revealed that *Narcissus assoanus* and *N. jonquilla* by sharing the emission of 2-butenoic acid, 3-methyl-, methyl ester, 3-Buten-1-ol, 3-methyl-, benzoic acid, methyl ester and other ester compound classes (isopentenoid and benzenoid) attracted similar insect visitors, moth (Sphingidae, Lepidoptera).

Elsewhere in Araceae, methyl benzoate was reported in *Anthurium lindenianum* (Kuanprasert et al. 1998) and *Anthurium armeniense* (Schwerdtfeger et al. 2002). Indole was detected in several *Arum* species (Kite 1995; Kite et al. 1998; Diaz and Kite 2002; Urru et al. 2010), *Amorphophallus eichleri* (Kite et al. 1998), and *Caladium bicolor* (Aiton) (Maia et al. 2012). In a field-trapping experiment *Psychoda* (Diptera, Psychodidae) species were attracted to indole (Kite et al. 1998). In *Sauromatum guttatum* (Borg-Karlson et al. 1994; Skubatz et al. 1996 but only in appendix), indole was detected. Large amount of indole together with *p*-cresol and *m*-cresol in the *S. guttatum* inflorescences most certainly contributes to its sapromyophilous character (Dobson 1994; Kite 1995).

It is possible that all the structures of inflorescence contribute in pollinator attraction, especially the appendix and spathe. Miyake and Yafuso (2003) tested different inflorescence structures and found the appendix of *Alocasia odora* is the main olfactory attractant. The

appendix was visited by the highest number of *Colocasiomyia* pollinators, followed by the staminate zone + upper sterile zone, and the pistillate zone + lower sterile zone (Miyake and Yafuso 2003). For *Homalomena* sp. without an appendix, Kumano and Yamaoka (2006) recorded that beetles (*Parastasia* and chrysomelids) were still more attracted to the spadix than the spathe. In this study, the spathe of *S. baangongensis* was recorded to release five floral compounds, inclusive of an additional N-containining compound, an indole. Although the spathe of a few aroid species (*Symplocarpus foetidus*, several *Arisaema* spp. are known to emit odour (Kevan 1989; Vogel and Martens 2000); the odour was rarely measured (except *Homalomena* by Kumano and Yamaoka 2006).

The arrival of *Colocasiomyia* flies and *Cycreon* beetles was correlated with the timing of the scent emission in *S. baangongensis* which started in the early morning and lasted for the next ca six hours. From Period I to Period II, only the pistillate zone increased the emission of total amount of floral scent, whereas floral scents from the other inflorescence structures were reduced. At this period, most insects (*Colocasiomyia* flies, *Cycreon* beetles, *Atheta* beetles) remained in the lower spathe chamber and increased the pollination probability. It is presumed that the increase of floral scent during this time maintained the insects in the lower chamber.

Conclusions

Anthesis of *S. baangongensis* begins at dawn and lasts ca 29 hours. The primary pollinators are *Colocasiomyia* flies and *Cycreon* beetles. *Chaloenus* beetles are inadvertent pollinators. The pollination mechanism of *S. baangongensis* is efficient with high reproductive success. Floral scent blends contain a dominant ester compound class, 3-butenoic acid, 3-methyl-, methyl ester, with the appendix as the main olfactory structure of the inflorescence. The floral biology, visiting insects, floral scents, reproductive systems, and insect pollen loads of *S. baangongensis* represents the first complete study of pollination biology in Schismatoglottideae.

Competing interests

The authors declare that they have no conflict of interest.

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Supplementary Material 1. Unidentified black (white arrows) and red ants (yellow arrows) are dispersing the fruits.

Supplementary Material 2. Pollen view under compound microscope. **a** *Schismatoglottis* pollen grains loaded on *Cycreon* beetles (200X magnification), **b–d** unidentified pollen grains on *Cycreon* beetles (200 x magnification), **e** *Schismatoglottis* pollen grains (100 x magnification), **f** unidentified pollen grain on *Colocasiomyia* flies (200X magnification).

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Table 1 Specific dates for each experiment

Experiment	Date
Flowering behaviour	26th – 30th October 2011
Insect capture	28th December 2009, 28th October 2011, 29th August 2013, 17th April 2014, 23rd June 2014, 5th August 2015, 5th September 2015, and 5th December 2015
Reproductive systems	Treatment 1: open pollination, between 21th November 2012 and 12th May 2015) Treatment 2: restricted access pollination, between 1st June 2014 and 12th June 2015 Treatment 3: cross pollination, conducted between 8th April 2015 and 8th May 2015 Treatment 4: autonomous self-pollination, conducted between 1st May 2014 and 30th July 2014
Pollen load	1st July 2014, 8th May 2015, 8th June 2015, and 23rd June 2015
Floral scent	3rd–4th February 2012, 30th August 2013, 1st September 2013, 3rd September 2013, 22nd May 2014

Table 2 Mean chemical composition (ng/hr), mean relative amount (%), and range for mean relative amount (%) of each VOC for whole inflorescence (inflo), appendix (app), pistillate zone (pis), spathe (spa) and staminate zone (sta) of *Schismatoglottis baangongensis*. Samples of whole inflorescence were trapped between 0530 h and 0800 h during pistillate phase of anthesis. Samples of individual floral structures were trapped twice, Period I (0600 h–0800 h) and Period II (0815 h –1015 h). N represents the number of replicates

Odour compounds	Retention time	Kovats index	inflo (N=6)	app ^{I} (N=2)	pis ^{I} (N=4)	spa ^{I} (N=3)	sta ^{I} (N=3)	app ^{II} (N=2)	pis ^{II} (N=2)	spa ^{II} (N=2)	sta ^{II} (N=4)
3-butenoic acid, 3-methyl-, methyl ester	5.635	800	87814.3 (99.4) (99.2-99.6)	4141.5 (77.6) (64 – 94.5)	535.2 (94) (93 – 95.6)	3182.0 (61) (55.9 – 70.3)	1785.1 (53.2) (47.4 – 61.2)	4181.2 (87.9) (77.4 – 93.4)	1006.0 (99.2) (99.3 – 99.9)	564.8 (67.7) (58.2 – 79.5)	2192.9 (95) (91.65 – 94)
2-butenoic acid, 3-methyl-, methyl ester	6.965	845	154.8 (0.2) (0.1-0.2)	7.1 (0.1) (0.1 – 0.2)	-	3.1 (0.1) (0.03 – 0.07)	-	7.5 (0.2) (0.1 – 0.2)	-	-	-
3-buten-1-ol, 3-methyl-	9.410	922	281.4 (0.3) (0.3-0.4)	7.9 (0.1) (0.1)	-	8.0 (0.1) (6.65 – 11.07)	-	11.3 (0.1) (0.1)	-	68.5 (8.2) (4 – 13.4)	-
Methyl benzoate	19.510	1270	27.0 (0.07) (0-0.2)	1210.7 (22.1) (5.2 – 35.7)	34.2 (6) (4.4 – 7)	1529.2 (29.3) (22 – 33.2)	1573.2 (46.8) (38.8 – 52.6)	561.3 (11.8) (6.3 – 22.5)	8.6 (0.8) (0.7 – 1.2)	83.3 (10) (7.1 – 12.3)	182.2 (5) (6 – 8.4)
Indole	35.750	1174	-	-	-	494.9 (9.5) (7.6 – 10.7)	-	-	-	225.0 (14.1) (25.45)	-
Total amount			88277.39	5467.2	569.5	5217.3	3358.3	4761.3	1014.6	941.6	2375.1

Table 3 Mean ± SD of pistillate flowers, fruits per infructescence, seeds per fruit and percentage of fruit set in open pollination, restricted access pollination, cross pollination, and autonomous self-pollination of *Schismatoglottis baangongensis.* Statistically distinct pairs of fruit set (%) are indicated with different letters; no significant difference was found for the number of seeds per fruit among the pollination treatments. N represents the number of replicates

Pollination treatments	Pistillate flowers	Fruits per infructescence	Seeds per fruit	Fruit set (%)
Open pollination	798 ± 55 (N = 26)	745 ± 79 (N = 26)	12 ± 3 (N = 100)	93 [a]
Restricted access pollination	-	700 ± 111 (N = 30)	11 ±1 (N = 100)	88 [a]
Cross pollination	-	285 ± 154 (N = 8)	8 ± 21 (N = 80)	36 [b]
Autonomous self-pollination	-	No fruit (N = 5)	-	0

Table 4 The mean ± SD (per inflorescence), range (per inflorescence), total number of visiting insects, and visitation frequency of *Schismatoglottis baangongensis* (N = 23). Statistically distinct pairs for each insect genera are indicated with different letters

	Atheta sp.	*Chaloenus* spp.	*Colocasiomyia* spp.	*Cycreon* sp.	*Parastasia* spp
lean ± SD	4 ± 3 [c]	4 ± 4 [c]	175 ± 108 [a]	33 ± 31 [b]	2 ± 2 [c]
ange	0 – 8	0 – 14	55 – 516	3 – 157	0 – 5
otal	43	86	4028	757	7
isitation frequency (%)	47.8	91.3	100	100	13
pecies	Unidentified to species level	*C. dohertyi* *C. latifrons* *C. schawalleri* *C.* sp. 1	*C.* aff. *bogneri*	Unidentified to species level	*P. gestroi* *P. nigripennis*

Table 5 The mean ± SD and range (per insect individual) of adhered pollen of *Schismatoglottis baangongensis* on insect visitors. Statistically distinct pairs for each insect genera are indicated with different letters

Pollen	*Atheta* sp. (N = 4)	*Chaloenus* spp. (N = 6)	*Colocasiomyia* spp. (N = 58)	*Cycreon* sp. (N = 52)
Mean ± SD	0 ± 0	71 ± 34 [b]	112 ± 99 [b]	1,827 ± 2,654 [a]
Range	0	0 – 100	8 – 496	12 – 11,700

Captions

Fig. 1 Flowering mechanisms and insect activities of *Schismatoglottis baangongensis*. Floral odour emits as early as 0400 on Day 1. Spathe opens at 0600 h coinciding with the arrival of insects. Insects starting to exit the inflorescence as early as 0500 h, Day 2 coinciding with the pollen release and shedding of spathe limb

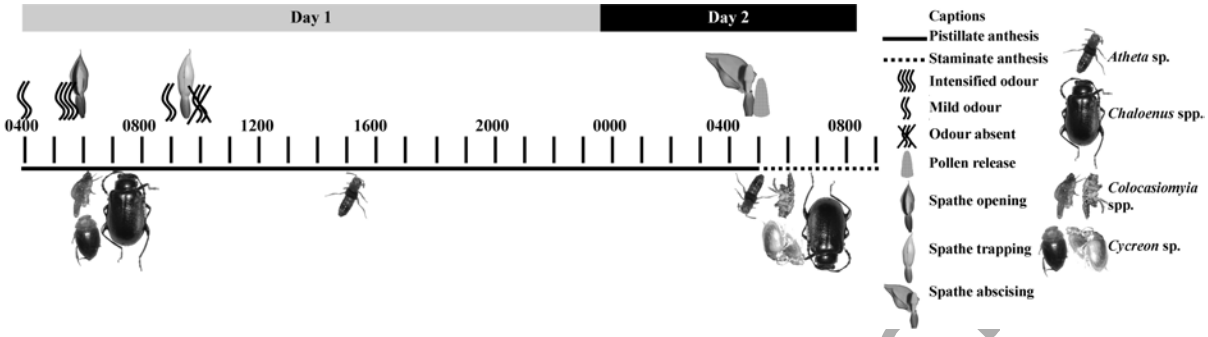

Fig. 2 Flowering mechanisms and insect activities of *Schismatoglottis baangongensis*. **a–e** flowering sequence: **a** a day prior to anthesis, **b & c** pistillate anthesis, **d** spathe abscising, **e** *Colocasiomyia* flies consuming the pollen and *Chaloenus* beetles remaining on appendix, **f** *Colocasiomyia* flies and *Cycreon* beetles consuming secretions on interpistillar staminodes, **g** *Chaloenus* beetle chewing interpistillar staminodes, **h** *Colocasiomyia* flies crawling onto *Chaloenus* beetle and transferred the adhered pollen to the *Chaloenus* beetle, **i** *Chaloenus* beetles laid eggs on appendix

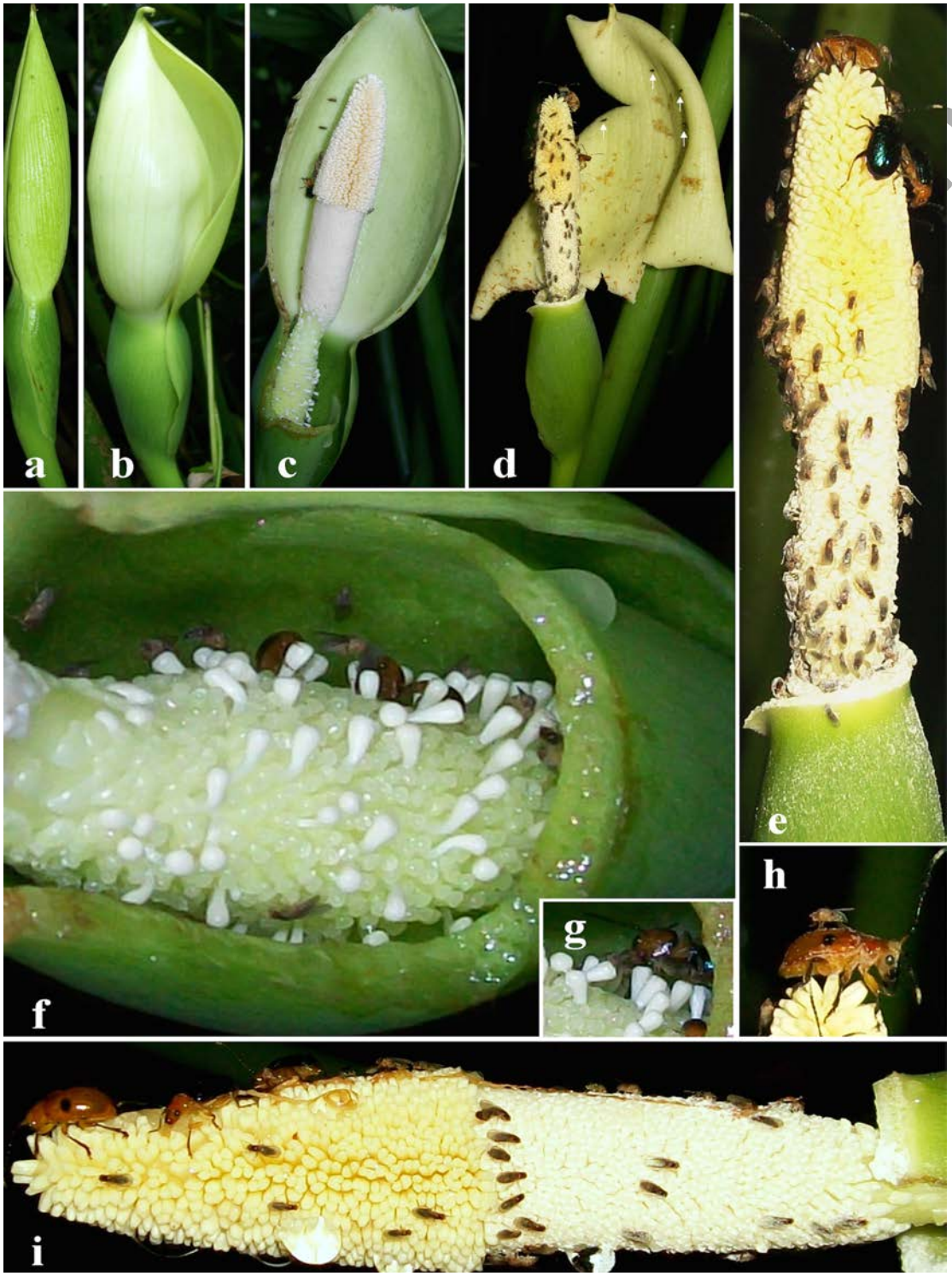

Fig. 3 Pollen load on visited insects of *S. baangongensis*. **a** *Schismatoglottis* pollen, **b** *Schismatoglottis* pollen load on *Colocasiomyia* flies, **c–d** *Schismatoglottis* pollen load on pronotum, elytra, tibia, femur and abdomen of *Cycreon* beetles