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Reproductive biology of two *Spathiphyllum* (Araceae) species in Los Tuxtlas, Veracruz, Mexico

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ABSTRACT

In the genus *Spathiphyllum* (Araceae), pollination studies and observations mainly suggest that male euglossine bees (Euglossini: Apidae) are the main pollinators, and *Spathiphyllum* species seem to fit perfectly in the perfume flower pollination syndrome. Other flower visitors, mainly stingless bees (Meliponini), are frequently observed but mostly regarded as pollen-thieves. Nevertheless, two previous investigations considered stingless bees to be major pollinators of two *Spathiphyllum* species, which raises the question of whether their role as pollinators has been underestimated so far. We conducted a comprehensive study on the reproductive biology of *Spathiphyllum croatii* and *S. ortgiesii*, two sympatric species in the region of Los Tuxtlas, Mexico, and investigated their flowering phenology, floral visitors, floral scents, floral (micro-)morphology, reproductive success, and their capability for self-pollination. *Spathiphyllum croatii* grew in large clusters at open sites, *S. ortgiesii* in small, scattered populations in the forest understory. Both species were protogynous and obligate outcrossers. Visits of male euglossine bees were rare (< 10%) and only observed at *S. croatii*. The main pollinators were pollen-collecting *Apis mellifera* (Apini) and *Plebeia* sp. (Meliponini; > 60% of all visits) in *S. croatii* and *Trigona fulviventris* (Meliponini; > 90%) in *S. ortgiesii*. The floral scent of the two species was distinct in its composition, intensity, and emission cycle. *Spathiphyllum croatii* had several dozen scent compounds, while the bouquet of *S. ortgiesii* consisted of only three compounds. The absence of a larger number of male euglossine visitors is remarkable, given that most of the major compounds in the scent of the two species are well-known male euglossine attractants. We conclude that our study species are specialized for pollination by pollen-collecting bees, and discuss potential reasons for the distinct pollinator spectra of the two plant species and the comparatively low abundance of euglossine bees as floral visitors.

1. Introduction

Araceae are characterized by their tiny flowers, arranged in compact spadices that are subtended by a mostly large bract, the spathe (Bown, 2000). Depending on the species, the flowers of the spadix are either unisexual or bisexual, but flowering is always protogynous. Thus, most species are obligate outcrossers (Mayo et al., 1997). In recent decades, the focus of research in aroid reproductive biology has been on taxa with unisexual flowers (e.g., *Dieffenbachia* and *Philodendron*; Gottsberger et al., 2013; Gibernau, 2015). These have a short flowering cycle, and pollination takes place in a pollination chamber, formed by the spathe, which encloses the inflorescence. Their pollinators are frequently beetles of the Dynastinae subfamily, which are lured by the floral scent that is synchronously emitted with the production of heat (thermogenesis) by the spadix at night. In this process, the scent is of particular importance since it specifically attracts the beetles and additionally triggers the movements of the pollinators between the plants (Seymour et al., 2003; Maia et al., 2012). Inside of the chamber, the beetles feed on diverse floral resources (exudates, floral tissue, pollen or sterile flowers) and benefit from the warm place as a shelter and mating site (Gottsberger and Amaral, 1984; Young, 1986; Gibernau and Barabé, 2002; Seymour et al., 2003; Maia et al., 2010; Gibernau, 2015).

Pollination studies of taxa with bisexual flowers (e.g., *Anthurium* and *Spathiphyllum*), are rather limited (Díaz Jiménez et al., 2019b). These

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plants frequently have long flowering cycles and their flowers are exposed (Croat, 1980; Montalvo and Ackerman, 1986; Hentrich et al., 2010). Similar to Araceae with unisexual flowers, the emission and composition of the floral scent seems to be of major importance for the attraction of pollinators (Williams and Dressler, 1976; Croat, 1980; Kuanprasert et al., 1998; Schwerdtfeger et al., 2002; Hentrich et al., 2010). Scent composition is rather diverse in this group. Many flowers produce sweet and pleasant scents to the human nose. Others have an unpleasant smell, resembling that of rotten fruits, and several are reported to be scentless (Croat, 1980; Kuanprasert et al., 1998; Schwerdtfeger et al., 2002). Some studies propose that the presence of compounds of a specific chemical class in the floral scent allows the prediction of a particular insect group as pollinators, e.g., fruit esters and/or alcohols – Drosophilidae; monoterpenes and/or simple aromatics in small numbers – male Euglossini; sesquiterpenes in higher quantities – nectar seeking bees (Gerlach and Schill, 1991; Schwerdtfeger et al., 2002). The specific attraction by scent compounds of a particular chemical class, in turn, promotes the specificity of pollinators and might act as pre-mating isolation mechanism (Williams and Dressler, 1976; Hentrich et al., 2010). A broad spectrum of pollinators has been proposed for Araceae species with bisexual flowers, including beetles, flies, bees, and even hummingbirds (Kraemer and Schmitt, 1999; Schwerdtfeger et al., 2002; Franz, 2007; Hentrich et al., 2007, 2010; Díaz Jiménez et al., 2019b).

The genus *Spathiphyllum* belongs to the early diverging Araceae subfamily Monsteroideae (Mayo et al., 1997). It comprises more than 89 species (Croat and Weessies, 2020) of which more than 90% are restricted to the Neotropics (Bunting, 1960; Mayo et al., 1997; Croat and Weessies, 2020). The plants are characterized by having a solitary inflorescence consisting of bisexual, protogynous flowers. The inflorescence sits on a long peduncle and bears a spathe at its base that is longer than the spadix (Bunting, 1960). The flowers of most plants emit a faint but pleasant floral scent. In the species studied so far, floral scent composition was similar to that of "perfume flowers" (Lewis et al., 1988; Gerlach and Schill, 1991; Schwerdtfeger et al., 2002; Hentrich et al., 2010), which are exclusively pollinated by male euglossine bees (Euglossini: Apidae) and only offer floral scent as a reward. Male euglossine bees are known to specifically collect volatile organic compounds from natural sources in forests, including the flowers of many plants, especially orchids (Dodson et al., 1969). Indeed, several anecdotal reports mention male euglossine bees as visitors or pollinators of Neotropical *Spathiphyllum* species (e.g., Vogel, 1963a; Dressler, 1967; Dodson et al., 1969). Williams and Dressler (1976) were the first to sum up observations of floral visitors of eight *Spathiphyllum* species at different localities. Seven species were pollinated by male euglossine bees that collected floral perfumes at the inflorescences, similar to their perfume gathering at "perfume flowers" (Vogel, 1963a; Dodson et al., 1969; Roubik and Hanson, 2004). The bees were attracted by the floral scent, and the attraction was highly specific with little overlap among the pollinating species of different *Spathiphyllum* species. Another study of the pollination of *S. humboldtii* showed that this species also specifically attracts two euglossine bee pollinators (Hentrich et al., 2010).

Besides male euglossine bees, stingless bees (Meliponini) have also been considered as pollinators of *Spathiphyllum* species, either alone (in *S. kalbreyeri*, Williams and Dressler, 1976), or in addition to euglossine bees (in *S. friedrichsthalii*, Montalvo and Ackerman, 1986). Stingless bees visited these plants to collect pollen from the male phase flowers. Therefore, pollination only took place when they landed on female phase inflorescences by accident, while searching for pollen. The floral scent composition of these species has never been published and is therefore unknown. In contrast to exclusively male euglossine-pollinated species, *S. friedrichsthalii* attracted many different euglossine species, which overall did not pollinate this plant species as effectively as stingless bees (Montalvo and Ackerman, 1986).

Altogether, reports of the high number of *Spathiphyllum* species that were mainly visited by male euglossine bees, in combination with the chemical composition of the species for which floral scents have been investigated so far, suggests that the genus is mainly pollinated by male euglossine bees and that stingless bee-pollination may rather be an exception. The pollination by male euglossine bees is especially interesting in the context of the sympatric occurrence of plant species. Due to the specific attraction of the pollinators, the composition of the floral scent has been considered to play an important role in the reproductive isolation of sympatric orchid and *Anthurium* species (Hills et al., 1972; Whitten and Williams, 1992; Hentrich et al., 2010). Since many *Spathiphyllum* species overlap in their distribution (Croat and Ortiz, in prep.), the genus is a good candidate to study this mechanism of reproductive isolation in sympatric populations.

In the Los Tuxtlas Biosphere Reserve (Veracruz, Mexico), three *Spathiphyllum* species grow sympatrically (Acebey and Krömer, 2008). We studied the reproductive biology of two of them, *Spathiphyllum croatii* Díaz Jim. & Pérez-Farr. and *S. ortgiesii* Regel. Although both species are distributed in the same region, they have different habitat preferences and growth habits. *Spathiphyllum croatii* forms large aggregations with dozens of plants along lakesides and streams, while the plants of *S. ortgiesii* occur dispersed in the forest (Díaz Jiménez et al., 2021). Nevertheless, the different habitats should not prevent euglossine bees to visit both *Spathiphyllum* species for potential pollinator-mediated gene flow since the plants grow in close proximity to each other and the bees are known to be good flyers that can cover large areas during their foraging flights (Janzen, 1971; Williams and Dodson, 1972; Roubik and Hanson, 2004). Our working-hypothesis was that the two *Spathiphyllum* species produce distinct floral scents that attract different male euglossine pollinators.

2. Materials and methods

2.1. Study sites

The study was conducted between January and December of 2014 at three sites of the region of Los Tuxtlas, Veracruz, Mexico. The first study site was located in a preserved area of tropical rainforest of the Los Tuxtlas Tropical Biological Station (EBTLT; 18°43′N, 95°25′W; 120–530 m asl). The other two sites present a significant degree of transformation for ecotourism activities and/or agricultural use: "Laguna Azul" is a riparian open area of a small lake surrounded by tropical rainforest to the north of the EBTLT (18°35 N, 95°05′W; 100–150 m asl); "La Palma" (18°33 N, 95°03′W; 24 m asl) is an area of swampy grassland (approximate linear distance between sites: EBTLT-Laguna Azul: 2 km; EBTLT-La Palma: 5 km; Laguna Azul-La Palma: 6 km; Fig. 1). The climate in the region is hot and humid; it is one of the five areas of Mexico where the annual rainfall exceeds 4000 mm (Guevara et al., 2004). The annual mean temperature varies between 24.1 and 27.2 °C (Gutiérrez-García and Ricker, 2011).

2.2. Study species

Spathiphyllum croatii Díaz Jim. & Pérez-Farr. is endemic to Mexico, while *S. ortgiesii* Regel is distributed in Mexico and Honduras (Acebey and Krömer, 2008; Díaz Jiménez et al., 2021). In the region of Los Tuxtlas, each species is found between 20 and 1000 m asl (Acebey and Krömer, 2008; Díaz Jiménez et al., 2021). *Spathiphyllum croatii* (studied at Laguna Azul and La Palma) grows mainly in open areas of disturbed and riparian vegetation (Fig. 2A). It forms large clusters of many plants (> 100). Individuals can reach up to 2.30 m in height. In contrast, *S. ortgiesii* (studied at the EBTLT and Laguna Azul) is less than one meter high and grows scattered in the understory (Fig. 2B) and on forest edges but is very common (P. Díaz Jiménez pers. obs.). Voucher specimens of both species were collected and deposited in the herbaria of EBTLT (Veracruz state) and MEXU (Mexico City; *P. Díaz J.* 1307, 1321).

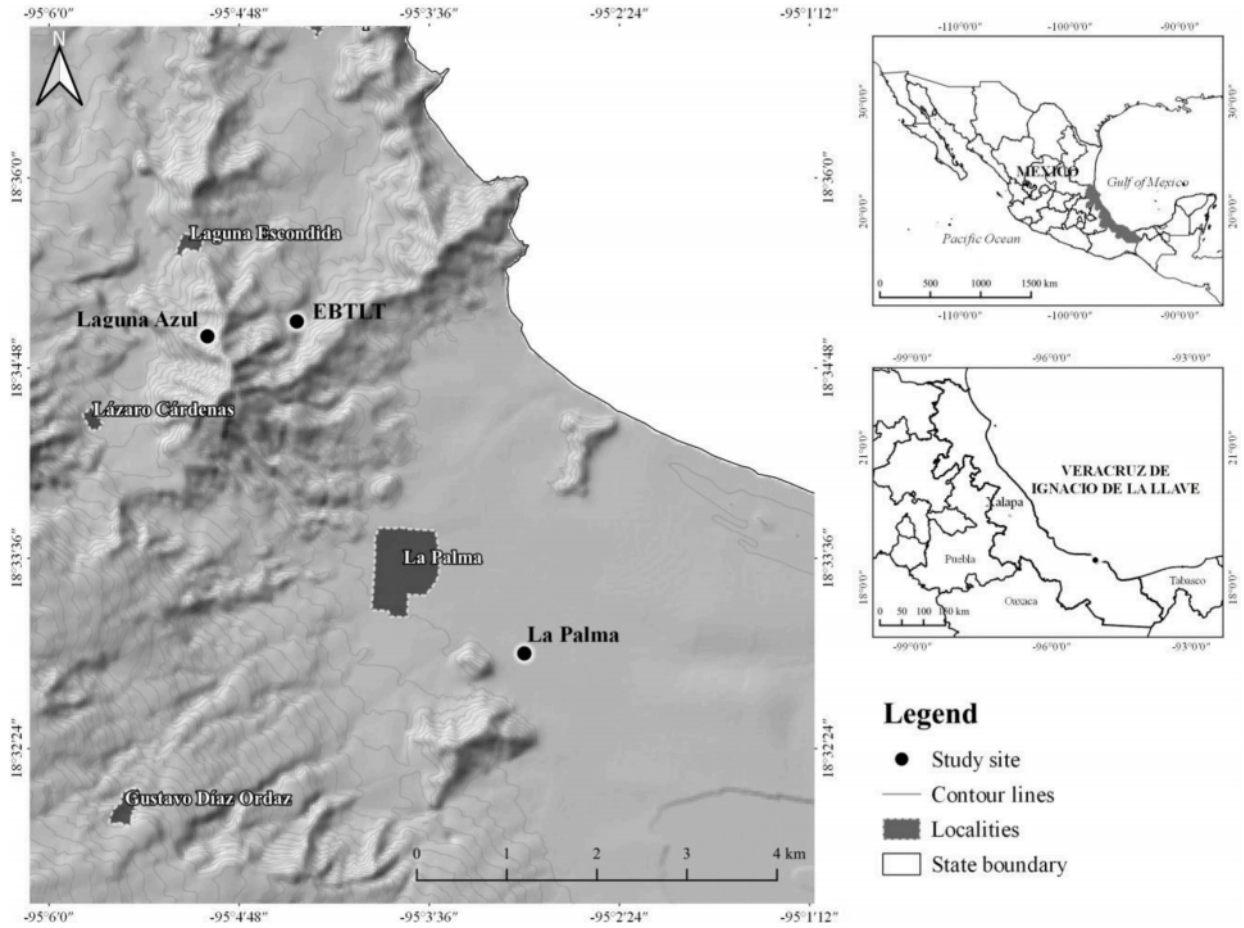

Fig. 1. Map showing the three study sites (black dots) at Los Tuxtlas region, Veracruz, Mexico. *Spathiphyllum croatii* was studied at Laguna Azul and La Palma, *S. ortgiesii* at Laguna Azul and EBTLT.

Fig. 2. Typical habitat of the study species. A: Large *Spathiphyllum croatii* population in riparian vegetation of the site Laguna Azul. B: Single plant of *S. ortgiesii* in forest understory of the site EBTLT.

2.3. Floral morphology

The length of the spadix of *S. croatii* ($n = 17$ spadices) and *S. ortgiesii* ($n = 9$ spadices) was measured with a millimeter ruler, and the number of flowers was counted manually. In addition, we measured the length of the styles of nine flowers each of seven inflorescences ($n = 63$ flowers) of *S. croatii*, and nine flowers each of nine inflorescences ($n = 81$ flowers) of *S. ortgiesii*, also with a millimeter ruler. A stereo microscope was used to determine the number of ovules of six flowers each of 21 inflorescences ($n = 126$ flowers) of *S. croatii*, and nine flowers each of nine inflorescences ($n = 81$ flowers) of *S. ortgiesii*.

For studying their micromorphology (surface structure of style, size and shape of pollen grains), five inflorescences of five individuals of each species were collected in the field (three in male phase and two in female phase). In addition, three inflorescences (two in female phase and one in male phase) of three individuals of *S. croatii* of the Botanical Garden of Nymphenburg (Munich, Germany) were used. The plant material was initially preserved in 70% ethanol. In the laboratory, the

inflorescences were cut into fragments, gradually dehydrated in propanol (70–100%), and dried in a BAL-TEC CPD 030 critical-point dryer (Balzers, Liechtenstein). Subsequently, the plant material was sputter-coated with gold (Balzers Union Sputter Coater, Balzers, Liechtenstein), and analyzed with a Zeiss DSM 942 (Zeiss, Oberkochen, Germany) and a FEI XL30 ESEM (FEI, Eindhoven, Netherlands) scanning electron microscope (SEM). Of the five inflorescences collected in the field, the size of 13 pollen grains of each species was measured under the microscopes.

The amount of pollen per flower was determined by counting the pollen grains per anther of each species ($n = 10$ anthers per species; two anthers from two randomly selected flowers each of five inflorescences) with a CASY TTC (Schärfe System GmbH, Reutlingen, Germany) cell counter and multiplying the results by four (total anther number per flower). Subsequently, pollen/ovule ratios (P/O; Cruden, 1977; Chouteau et al., 2006) were calculated by dividing the mean of sums of pollen grains per flower by the mean of ovules per flower.

2.4. Flowering phenology

Sporadic observations of the flowering status of randomly selected individuals of the study species at all three study sites were made monthly throughout 2014. In March, 43 *S. croatii* plants in La Palma and 64 *S. ortgiesii* plants in EBTLT were tagged before any inflorescences were visible. During the main flowering time, between May and June, the flowering phenology of the tagged plants was studied in more detail on a daily basis: the start of the anthesis (female phase) was identified by testing stigmatic receptivity with H_2O_2 (Galen and Plowright, 1987) and by determining the presence of floral scent by smelling the inflorescences with our nose (Schwerdtfeger et al., 2002); the duration of the sexual phases was assessed by examining the stigmas turning from a moist-transparent to a dry-brownish appearance (end of female phase; Hentrich et al., 2010), and by documenting the emergence of the first (beginning of male phase) and the last anthers of the inflorescence (end of male phase and end of flowering).

2.5. Effect of floral visits on fruit set

In order to find out if the flower visitors are successful pollinators and to exclude the possibility of fruit set by apomixis or autonomous self-pollination, we covered immature inflorescences of *S. croatii* ($n = 14$) and *S. ortgiesii* ($n = 14$) with a cloth (mesh size < 1 mm) until the end of anthesis. The cloth was fixed on a wire structure and tied with yarn. Subsequently, fruit development was recorded between the second and third month after bagging and fruits and seeds per infructescence were counted. Simultaneously, inflorescences of our visitor observations (*S. croatii:* $n = 14$, *S. ortgiesii:* $n = 20$) were used to count fruits after fruit development. These inflorescences remained uncovered for the entire flowering period.

2.6. Visitor observations

In both species we made exploratory observations (directly or by video recordings; JVC Everio GZ-MG50 (JVC KENWOOD Corporation, USA); Sony DCR-SR65 night vision camera (Sony Corporation, Japan)) between 14 and 16 May 2014 (06:00–18:00 h and 21:00–01:00 h), to determine the floral visitor spectra and visiting time, and to collect voucher specimens of the visitors. Based on these visiting times, we defined the periods of all further observations. Floral visitors in both sexual phases of 14 different inflorescences (seven each in the female and male phase) of *S. croatii* were observed at Laguna Azul (20 July-01 September 2014; continuously from 07:00–11:00 h), and of 20 different inflorescences in *S. ortgiesii* (continuously from 08:00–14:00 h), thereof ten (five each in the female and male phase) at EBTLT (14 June-08 July 2014) and ten (five each in the female and male phase) at Laguna Azul (20 July-03 September 2014) by one person, respectively. In total, we conducted 56 h of observations in seven days in *S. croatii* and 140 h in 12 days in *S. ortgiesii*. For each floral visitor, we annotated species or morphospecies identity, time and duration of the visit, behavior, and anthesis phase. Based on their behavior, the occurrence of visits to inflorescences of both sexual phases, the presence of pollen on their bodies, as well as contact with the stigmas, insects were categorized as visitors or pollinators. Some floral visitors were also collected during the observations for further identification in the laboratory. Euglossini were identified by Dr. Günter Gerlach (Nymphenburg Botanical Garden, Germany), Meliponini by Dr. Stefan Jarau (Pädagogische Hochschule Vorarlberg, Austria), and Halictidae by Dr. Ismael Alejandro Hinojosa Díaz (UNAM, Mexico). Other visiting insects were identified at the order or family level, using the guide of Borror and White (1970). Vouchers of the insect species were deposited in the entomological collection of the EBTLT (*P. Díaz J. 01, 02, 03, 04, 05, 12, 13, 15, 16*).

2.7. Floral scent analysis

Fluctuation in scent emission throughout the day was determined for both plant species by smelling the inflorescence with the nose. Presence and intensity (two categories: weak or strong) of the floral scent were noted. This was done for several days at hourly intervals during day and night. Additionally, floral scent was collected between the first and fourth day of the female or male phase of anthesis, respectively, of different or the same inflorescences, using the "dynamic headspace" method (Hills and Schutzman, 1990). In *S. croatii*, samples of five individuals (n samples = 6; two female, two male, and one of each in both phases) in Laguna Azul (22 July-03 September 2014; 07:00–10:00 h), and of six individuals (n samples = 10; one female, one male, and four of each in both phases) in La Palma (28 May and 21 July 2014; 07:00–10:00 h) were collected. In *S. ortgiesii*, we sampled five individuals (n samples = 7; three female and two of each in both phases) in EBTLT (09–20 June 2014; 11:00–14:00 h) and three individuals (n samples = 5; one female and two of each in both phases) in Laguna Azul (23 July-05 September 2014; 11:00–14:00 h).

Each inflorescence was enclosed by an inert oven bag (Toppits, Cofresco). Two holes were cut in the bag. In one hole, a glass tube filled with activated charcoal, fixed by glass wool plugs, was placed to clean the incoming air. An absorbent tube filled with 25 mg of Tenax TA (mesh 80–100, Macherey Nagel, Düren, Germany) and 40 mg of Carbopack B (mesh 20–40, Supelco, Bellefonte, PA, USA), fixed by glass wool plugs, was placed in the second hole. The absorbent tube was connected to a battery-operated rotary vane pump (G12/01–4 EB; Gardner Denver Thomas GmbH, Memmingen, Germany) that drew air for three hours at a flow rate of 250 ml/min. The captured volatiles were eluted with 0.2 ml of acetone (Rotisolv, purity > 99.99%, Carl Roth, Karlsruhe, Germany) and placed into glass vials with PTFE-lined screw caps (11.6 × 32 mm; Macherey Nagel, Düren, Germany). Using the methods described above, we took control samples from empty oven bags, placed at 1.50 m distance to the inflorescence simultaneously with the plant samples to discriminate between plant and ambient scent compounds (one in La Palma; two in Laguna Azul; one in EBTLT).

The samples were analyzed on a gas chromatograph coupled to a mass spectrometer (Shimadzu GC–MS-QP2010 Ultra) and equipped with an AOC-20i autoinjector (Shimadzu, Tokyo, Japan) as well as a ZB-5 fused silica column (5% phenyl polysiloxane; 30 m long, inner diameter 0.32 mm, film thickness 0.25 μm, Phenomenex). Of each sample, 1 μl was injected (injection temperature: 220 °C; split ratio: 1:1), and the column flow (carrier gas: helium) was set at 3 ml min^{-1}. As the GC–MS system is also equipped with an electroantennographic detection system, the column is split at the end (AFT splitter package, Shimadzu), and one third of the effluent is transferred to the mass spectrometer. The GC oven temperature was held for 1 min at 40 °C, then increased by 10 °C per min to 220 °C, and held for 2 min. The MS interface worked at 220 °C and the ion source at 200 °C. Mass spectra were taken at 70 eV (in EI mode) from m/z 30 to 350.

Samples of the floral perfumes were processed using the GCMSolution package, Version 2.72 (Shimadzu Corporation). Components were (tentatively) identified by a combination of comparison of Kováts' retention indices based on a series of *n*-alkanes (C_7–C_{20}) and mass spectra, to data available in various data bases [Adams (2007), FFNSC 2, W9N11, ESSENTIAL OILS (available in MassFinder 3)]. Whenever possible, components were verified by comparison to authentic reference standards available in the Plant Ecology Lab of the Paris-Lodron-University of Salzburg. Percentages of peak areas of each compound obtained from each sample were calculated.

2.8. Statistical analysis

To compare the duration of visits (data were not normally distributed) to inflorescences of different sexual phases of both aroid species, we calculated per species pairwise Euclidean distances in visitation time among all observations and analyzed the data using univariate PERMANOVA analyses (one per plant species; plant sex and bee taxa as fixed factors in crossed designs; 10,000 permutations) in Primer 7.0.13 with the add-on package Permanova+1. Given that the designs were unbalanced (different replicates per group), we used Type III Sum of squares to test which factors significantly contributed to the full model.

Similarities and dissimilarities of floral bouquets among samples were visualized using non-metric multidimensional scaling (NMDS) in Primer 7.0.13 (Clarke and Gorley, 2001). The NMDS plot was based on a triangular similarity matrix calculated from the standardized (by total peak area), log-transformed fragrance data using the Bray–Curtis similarity index (Clarke and Gorley, 2001). In ideal MDS plots, the rank order of distances between samples corresponds exactly to the ranked similarities in the similarity matrix. Deviations from an exact match between the similarity matrix and the MDS plot are expressed in terms of 'stress', with values < 0.05 indicating an excellent representation of the data (Clarke and Warwick, 2001).

A one-way analysis of similarity (ANOSIM) based on the Bray-Curtis similarities was used (999 permutations) to test the null hypothesis that there is no difference in relative scent composition between the two species. ANOSIM is a non-parametric distribution-free analogue of a one-way ANOVA (Clarke and Green, 1988). Subsequently, the means of similarity in floral fragrance composition within species and compounds responsible for the observed dissimilarity pattern were identified using the similarity percentage analysis (SIMPER) in Primer 7.0.13 (Clarke and Warwick, 2001). Due to the limited power, we did not test for differences in scent between female and male stages within species.

3. Results

3.1. Floral morphology

The flowers, cream-yellowish in *S. croatii* and greenish-whitish in *S. ortgiesii*, were grouped on a cylindrical spadix that had a median length of 8 cm (range: 3.6–12.2 cm; $n = 17$) and 6 cm (range: 5–12 cm; $n = 9$), respectively (Fig. 3A, C). *S. croatii* presented a median of 139 flowers per inflorescence (range: 93–351, $n = 17$), whereas *S. ortgiesii* had 171 (range: 131–297; $n = 9$). Both species had four or six inconspicuous tepals, four stamens with short filaments, and one style with a median length of 5 mm (range: 4–6 mm; $n = 63$ flowers) in *S. croatii* and 3 mm (range: 2–4 mm; $n = 81$ flowers) in *S. ortgiesii*. Epidermal cells of the style of *S. croatii* were longitudinally stretched and had a smooth surface, while they were more or less isodiametric and showed a folded surface in *S. ortgiesii* (Fig. 3B, D). On average, we counted more pollen

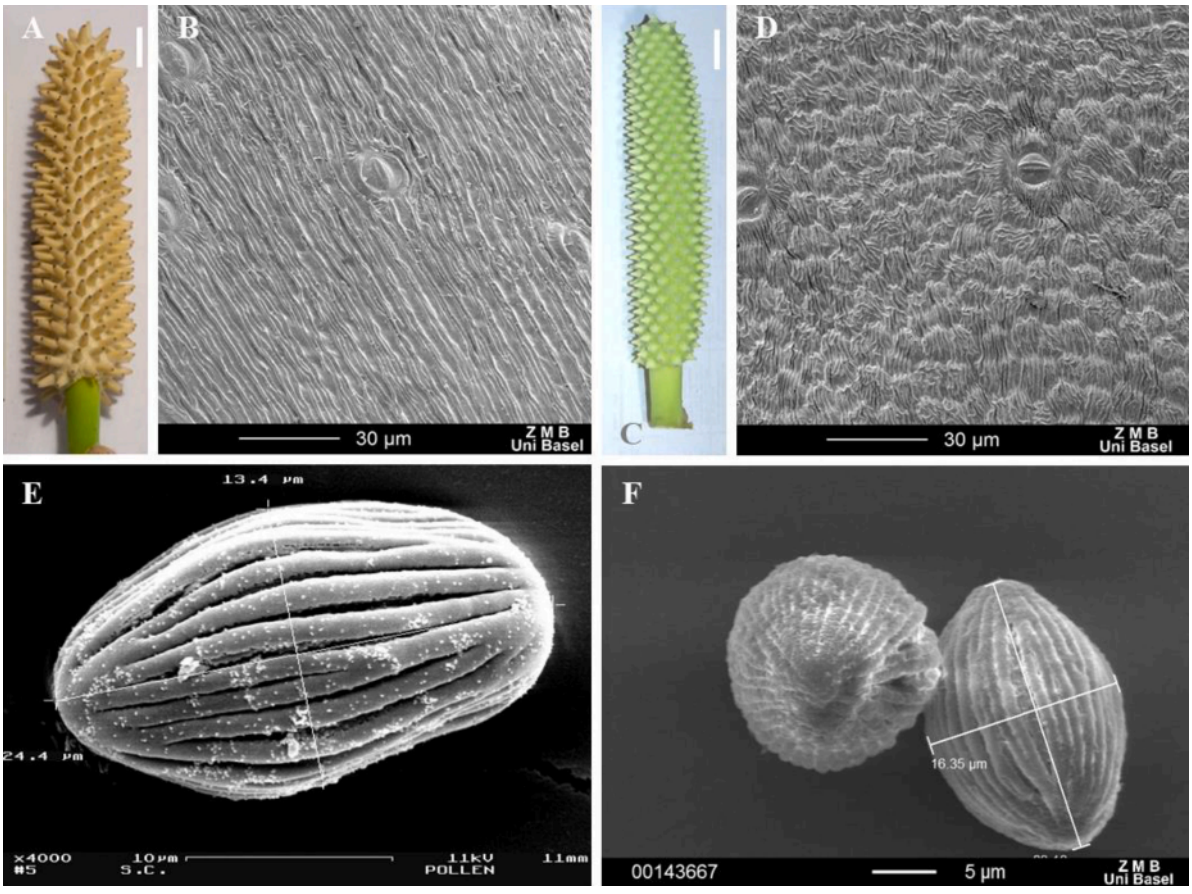

Fig. 3. Inflorescences and floral microstructures of *Spathiphyllum croatii* (A, B, E) and *S. ortgiesii* (C, D, F). A: Inflorescence in male phase; C: Inflorescence in female phase; B, D: epidermis of the style; E, F: pollen grains. Scale bar at inflorescence photographs = 1 cm.

grains per flower in *S. croatii* (95,060 ± 25,438) than in *S. ortgiesii* (53,420 ± 13,524; Table 1). Multiplied by the median sum of flowers per inflorescence, the average number of pollen grains per inflorescence was also higher in *S. croatii* than in *S. ortgiesii* (13,213,340 vs. 9134,820). The pollen grains of both species looked similar – they had spheroidal, elliptic, plicate monads without pollen coating and without aperture (Fig. 3E, F). In *S. croatii*, pollen grains were 23.3 μm long (range: 19.9–24.5 μm) and 15 μm wide (range: 13.1–16.4 μm, $n = 13$), whereas they were 21.15 μm long (range: 17.28–25.3 μm) and 17.09 μm wide (range: 15.12–19.9 μm, $n = 13$) in *S. ortgiesii* (Table 1). None of the pollen grains were mixed with calcium oxalate crystals. The average number of ovules per flower was also higher in *S. croatii* (11.64 ± 2.17) than in *S. ortgiesii* (6.08 ± 1.29), and the resulting P/O ratio in both species was at about 8000 (Table 1).

3.2. Flowering phenology

The two species exhibited different phenological patterns. Many individuals of *S. croatii* flowered in a short period of time, whereas only few plants flowered at a time in *S. ortgiesii* (Fig. 4). All marked individuals of *S. croatii* ($n = 43$) flowered between the beginning of May and the beginning of June. The emergence of inflorescences was synchronous among the majority of the individuals. After the flowering peak between May and June, other individuals of the same and other populations produced a constantly low number of new inflorescences from June to December. Few flowering plants were observed between January and April. In contrast to *S. croatii*, only some of the marked individuals (20 out of 64) of *S. ortgiesii* flowered. The inflorescences emerged asynchronously among individuals in a period between May and June. There was no clearly defined flowering peak in this species, and little flowering in marked and unmarked plants was consistently observed from July to December. No inflorescences at all were observed from January to April. All flowering tagged plants of both species produced only a single inflorescence per individual in the entire year.

The floral cycle of *S. croatii* began with the simultaneous opening of the spathe, the emission of a strong, pleasant aroma, and the receptivity of all stigmas of the spadix (beginning of the female phase). In *S. ortgiesii*, anthesis began on the third or fourth day after the spathe had opened. Similar to *S. croatii*, all stigmas of the spadix were receptive at once and receptivity coincided with the emission of scent. However, the scent was weak. In neither species pollination drops were observed on the stigmas. The female phase ended after six days in *S. croatii* (range: 3–10 d; $n = 43$) and eight days in *S. ortgiesii* (range: 5–13 d; $n = 20$) with the withering of all stigmas of an inflorescence in the evening. The male phase began the next morning with the emergence and opening of the first anthers. There was no overlap between the two phases and no intermediate phase was present in the inflorescences of the same individual in either of the species. Emergence and maturation of the anthers occurred in a transition from the base to the tip of the spadix in both species, and flowering ended after another 18 days in *S. croatii* (range: 16–22 d; $n = 43$) and 24 days in *S. ortgiesii* (range: 18–30; $n = 20$) with the withering of the last anthers. In *S. croatii*, the anthers emerged simultaneously with the beginning of scent emission. In *S. ortgiesii*, the anthers emerged up to four hours before scent emission. Due to the longer male flowering phase in both species, there were three times as many inflorescences in the populations in the male phase than in the female phase.

Table 1
Number of pollen grains per anther and flower, size of pollen grains, number of ovules per flower, and P/O ratios of *Spathiphyllum croatii* and *S. ortgiesii*. Values are given as average ± SD or median and range (R).

	Spathiphyllum croatii		N	*Spathiphyllum ortgiesii*		N
pollen grains per anther	23,765 ± 6359		10	13,355 ± 3381		10
pollen grains per flower	95,060 ± 25,438		10	53,420 ± 13,524		10
ovules per flower	11.64 ± 2.17		126	6.08 ± 1.29		81
P/O ratio	8164			8773		
	Median	**R**		**Median**	**R**	
length of pollen grains (μm)	23.3	19.9–24.5	13	21.15	17.28–25.3	13
width of pollen grains (μm)	15	13.1–16.4	13	17.09	15.12–19.9	13

3.3. Effect of floral visits on fruit set

From the covered inflorescences, only five of 14 inflorescences of *S. croatii* produced some fruit, with an average of 1.4 (± 0.54) fruits per inflorescence (0.72–1.44% of the average number of flowers of an inflorescence). Each fruit contained an average of 3.85 (± 2.41) seeds (8–67% of the average number of ovules per flower). All other covered inflorescences dried out at the end of the male phase and fell off without producing any fruit. In *S. ortgiesii*, all covered inflorescences withered at the end of flowering and did not produce any fruit. In contrast, in all uncovered inflorescences of both *Spathiphyllum* species studied, which were visited by different bee species, every single flower produced a fruit so that the fruit development was 100% for each infructescence.

3.4. Visitor observations

3.4.1. Spathiphyllum croatii

In total we recorded 324 visits to *S. croatii* with female bees of the species *Plebeia* sp. (Meliponini) and *Apis mellifera* (Apini) being the main flower visitors (> 60%; Table 2; Fig. 5A, B). Other female bees that visited the inflorescences in lower frequencies were *Partamona bilineata, Trigona nigerrima*, and *T. fulviventris* (Meliponini). Furthermore, few male *Euglossa viridissima* and *E. mixta* visited the flowers (2% in the female phase and 8% in the male phase for *E. viridissima*, and less than 1% in the male phase for *E. mixta*). The majority of the bees arrived between 07:45 and 10:14 h, with an average of two visits per 15 min/inflorescence (Fig. 6A).

Stingless bees and honeybees visited the male inflorescences to collect pollen. Except for *Plebeia* sp., they all landed on the tips of the styles, crawled on them over the spadix, and stopped from time to time to collect pollen from the open anthers below them by using their front legs. Additionally, some bee individuals also ate pollen. Due to its small size, *Plebeia* sp. did not only crawl on the tips of the styles after landing, but also between them to move over the spadix (Fig. 5A). All bees frequently interrupted pollen-collecting to fly up and transfer pollen to their corbiculae. Afterwards, they returned to the same inflorescence and continued pollen-collecting. This behavior was repeated several times before they stopped and flew to another nearby inflorescence. Besides male phase inflorescences, the bees also landed on female phase ones that were in their vicinity. Here, they moved quickly over the spadix by crawling on or between (*Plebeia* sp.) the styles. Thereby, pollen masses stored in their corbiculae came into contact with the receptive stigmas. We did not observe the bees collecting, eating or drinking anything at the female phase flowers. From time to time, they also flew up and landed on the same female phase inflorescence, but this behavior was repeated less often than in male phase ones, sometimes only once. Generally, after a short time, they flew to another nearby inflorescence without returning to the same female phase inflorescence. All pollen-collecting bees landed more often on male phase (87%) than on female phase inflorescences. Both, the phase of the inflorescence (PERMANOVA: pseudo-$F_{df=1300} = 19.92$; $p < 0.001$) as well as the bee species (PERMANOVA: pseudo-$F_{df=4300} = 18.50$; $p < 0.001$) had a significant effect on the duration of the visits. These effects were independent of each other (plant sex × bee species; PERMANOVA: pseudo-$F_{df=4300} = 1.38$; $p = 0.23$). For most bee species, visits to male phase inflorescences were longer than to female phase ones (Table 2). Different bee species, however, generally diverged in the time they stayed at inflorescences of

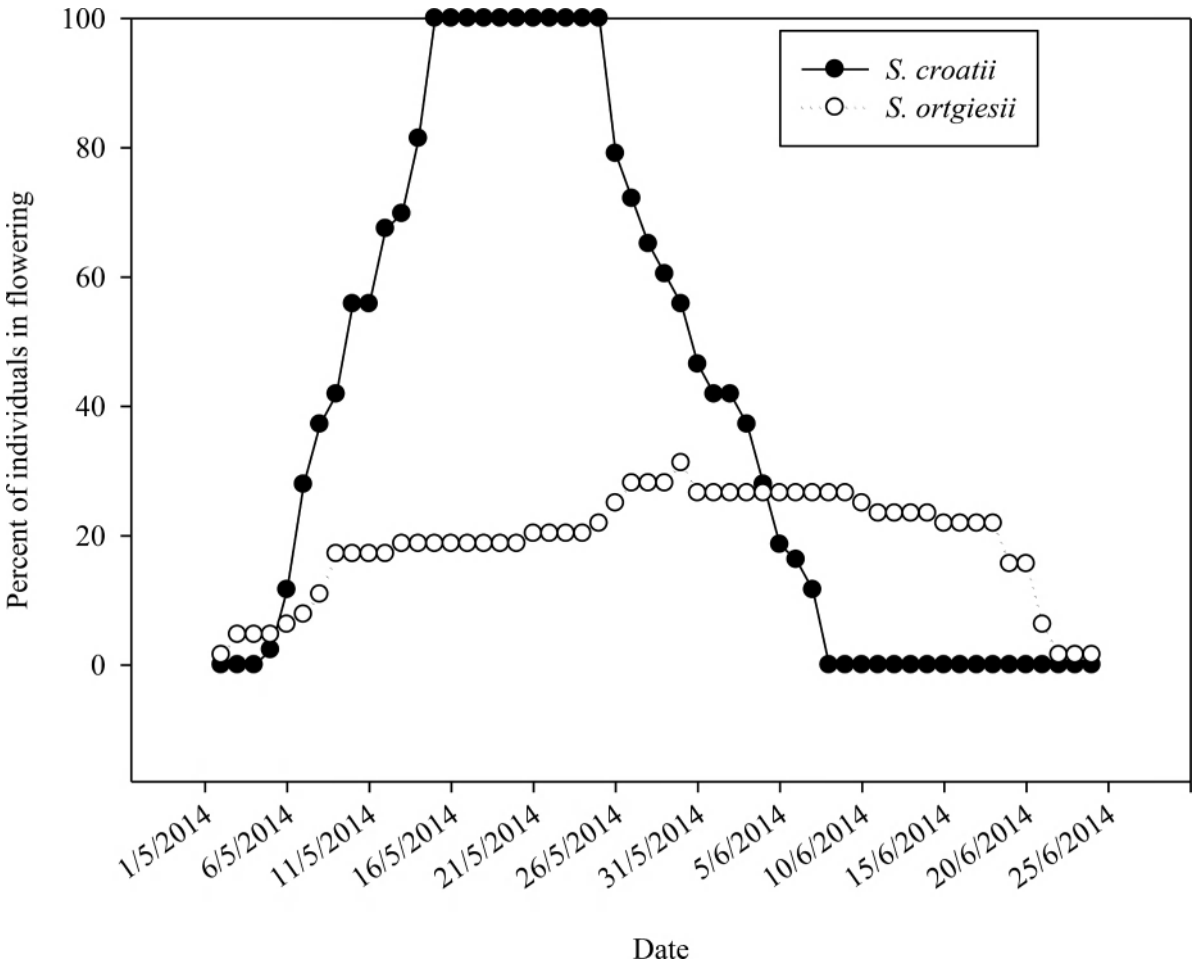

Fig. 4. Percentage of flowering individuals of *Spathiphyllum croatii* ($n = 43$) and *S. ortgiesii* ($n = 64$) recorded between May 1 and June 25, 2014.

S. croatii. For example, the visiting behavior of *T. fulviventris* was similar to that of the other pollen-collecting bees, but differed in that they moved and flew much more slowly. Furthermore, when visiting a female phase inflorescence, they occasionally remained immobile for a few seconds, giving the impression of being very attentive before they continued crawling. Therefore, the time they spent on female phase inflorescences was usually higher than that of the main visiting species (Table 2). They also flew up from time to time, but in contrast to the other pollen-collecting bees, they repeated this behavior in most cases as often as in male phase inflorescences.

Euglossa viridissima also visited male- and female phase inflorescences, but showed a different behavior than the pollen-collecting bees: after landing, they crawled over the spadix on the tips of the styles and wiped the surface of the base of the styles with the tarsal brushes of their forelegs (Fig. 5C). Subsequently, they flew up, hovered in front of the inflorescence, and landed again on the same inflorescence. This behavior was repeated several times and was identical on male- and female phase inflorescences. While moving over the flowers and during the wiping-movements, their legs touched the anthers in male phase, and the stigmas in female phase flowers. In general, the visits of *E. viridissima* lasted considerably longer than those of all other bee species ($p < 0.03$ in PERMANOVA post-hoc analyses) and were almost as long on female phase inflorescences as on the male phase ones (Table 2). At the end of a visit, these bees flew away quickly in any direction. Interestingly, *E. viridissima* was the only visitor that was observed in days with moderate rain. However, all *E. viridissima* observed in the rain only visited hanging inflorescences, where the spadix practically served as an umbrella over the spadix. *Euglossa mixta* was only observed once at a male inflorescence. It landed on the spadix without wiping the styles and flew away after a few seconds (Table 2). Apart from the different bee species, one hemipteran was observed crawling for a few seconds over male inflorescences without eating anything.

3.4.2. *Spathiphyllum ortgiesii*

In *S. ortgiesii*, a total of 352 visits were recorded. Female *Trigona fulviventris* bees (Meliponini) were the main flower visitors accounting for more than 90% of all visits (Table 2; Fig. 5D). Besides this species, a low number of female *Caenaugochlora* sp. bees was observed. Visits took place much later in the day than in *S. croatii*, with a peak between 11:15 and 13:29 h, and with an average frequency of 1.5 visits per 15 min/inflorescence (Fig. 6B).

Both bee species showed the same behavior as the pollen-collecting bees in *S. croatii. Trigona fulviventris* arrived at the inflorescences irrespective of whether the flowers were fragrant to us or not (85% scented vs. 15% non-scented). When visiting female inflorescences and crawling on the tips of the styles, pollen masses stored in the hind tibia of *T. fulviventris* made contact with the receptive stigmas of the flowers (Fig. 5D). Unlike *T. fulviventris, Caenaugochlora* sp. visited only fragrant inflorescences. They also crawled on the tips of the styles or between them, and masses of pollen stored in the tibia made contact with the receptive stigmas. However, they always moved faster on the inflorescences and therefore stayed for a shorter period of time than *T. fulviventris*. Again, neither visitor species was observed collecting, eating or drinking anything at the female phase flowers. Similar to the observations in *S. croatii*, both bee species landed more often on male phase (73%) than on female phase inflorescences and all visits to male

Table 2
Floral visitors of *Spathiphyllum croatii* and *S. ortgiesii*. Absolute numbers (N), proportions of visits for all observations of each plant species (%), and mean visiting time ± SD (T) are given for each phase of anthesis (*F* = female, *M* = male), respectively. The sex for each species of bee is given (* = female, ** = male).

Floral visitor	*Spathiphyllum croatii*			*Spathiphyllum ortgiesii*		
	N	%	T [s]	N	%	T [s]
Apini						
*Apis mellifera**	11 (F) 86 (M)	3 27	6 ± 5 73 ± 76			
Meliponini						
Plebeia sp.*	13 (F) 92 (M)	4 28	6 ± 5 139 ± 104			
*Partamona bilineata**	5 (F) 47 (M)	2 15	9 ± 7 67 ± 49			
*Trigona nigerrima**	4 (F) 21 (M)	1 6	25 ± 14 217 ± 133			
*Trigona fulviventris**	3 (F) 2 (M)	1 1	23 ± 25 68 ± 69	86 (F) 245 (M)	24 70	107 ± 122 123 ± 112
Euglossini						
*Euglossa viridissima***	7 (F) 25 (M)	2 8	274 ± 329 319 ± 255			
*Euglossa mixta***	1 (M)		3			
Halictidae						
Caenaugochlora sp.*				6 (F) 5 (M)	2 1	17 ± 13 166 ± 111
Halictidae 1*	4 (F)		4 ± 1			
Halictidae 2*	1 (F)		9			
Others						
Chrysomelidae				6 (M)		15 ± 753
Curculionidae				1 (M)		4
Nitidulidae				3 (M)		4 ± 898
Hemiptera	2 (M)		6 ± 3			
Total	324	97		352	97	

phase inflorescences were longer than to female phases ones (PERMANOVA: pseudo-$F_{df=1328} = 5.50$; $p = 0.03$), but now with overall similar visitation times of the different bee species (*T. fulviventris, Caenaugochlora* sp.; PERMANOVA: pseudo-$F_{df=1328} = 0.44$; $p = 0.48$). As in *S. croatii* the effect of sexual phase of the plant was independent of bee species (plant sex × bee species; PERMANOVA: pseudo-$F_{df=1328} = 3.53$; $p = 0.06$). Although, conspecific flowering plants were generally more widely separated than in *S. croatii*, we frequently observed *T. fulviventris* flying directly to inflorescences standing 3–5 m (sometimes up to 10 m) in the vicinity of the ones they visited before. Besides the bees, we observed small numbers of different beetles at male phase inflorescences (Table 2), which ate pollen. Chrysomelidae also bit the styles and occasionally cut the upper part off partially or completely.

3.5. Floral scent analysis

On all days of the female and male phases, the spadices of *S. croatii* emitted a strong and pleasant scent to the human nose between 07:00 and 11:00 h. The fragrance of *S. ortgiesii* was weak and perceptible to us only between 11:00 and 14:00 h during the female phase and weaker during the first two to three days of the male phase. On subsequent days of the male phase the fragrance was imperceptible. During the night, the odor was imperceptible for us in both species.

A total of 49 compounds were identified in the floral scent of *S. croatii*, three in *S. ortgiesii* (Appendix A, B). The dominant chemical compound classes were terpenoids (median% total peak area in female phase samples = 25%, male phase = 33%) and aromatics (31% and 55% respectively) in *S. croatii* (Appendix A). The main compounds (> 2% in more than 50% of samples) in this species were: (*E,E*)-α-Farnesene (15% and 19% respectively), (*E*)-α-Farnesene epoxide (12% and 19%), Methyl benzoate (1% and 6%), Methyl salicylate (18% and 9%), Phenylacetonitrile (4% and 3%), and two unknown compounds (3% and 2%, 2% and 1% respectively; Table 3; see also Appendix D). *Spathiphyllum ortgiesii* had a very simple scent profile, with just three compounds detected. 2-Phenylethanol (93% and 50%) and Isoamyl alcohol (5% and 34%) dominated the scent in both sexual phases, while β-Myrcene (1% and 16%) was only present in lower quantities (Table 3; see also Appendix E). *β*-Myrcene and 2-Phenylethanol were shared in both species (Appendix B; Table 3).

NMDS visualization produced clearly species-specific clusters for the fragrance samples, with a stress of 0.01 (Appendix C), and there were significant differences in floral fragrance composition among species (ANOSIM: $R = 1$, $P < 0.1$). The compounds that mainly contributed to dissimilarities were all the major compounds found in each of the species (Table 3).

4. Discussion

Our results show that pollen-collecting, female stingless bees and honeybees dominated the visits in the *Spathiphyllum* species studied. The bees landed on inflorescences of both sexual phases and transferred pollen to the stigmas while moving over the flowers. In contrast to covered inflorescences, which produced no (*S. ortgiesii*) or almost no fruits (*S. croatii*), naturally pollinated inflorescences showed 100% fruit set. We therefore assume that fruit production in both plant species results from pollination by the documented visiting bees and not by autogamous pollination or apomixis.

Spathiphyllum ortgiesii was unique in terms of its pollination biology compared to *S. croatii* and all other *Spathiphyllum* species investigated so far. The species was highly specific in its pollinator spectrum, and the main and almost only pollinator observed was *Trigona fulviventris*, which visited the flowers in low frequency, but regularly. A striking peculiarity of the flower visits of *T. fulviventris* was that the bee stayed longer at female inflorescences than the stingless bees at *S. croatii* and *S. friedrichsthalii* (Montalvo and Ackerman, 1986) and that the visiting time was almost the same for female and male phase inflorescences. During our observations, we did not notice any *T. fulviventris* at the female phase flowers that indicated the collection of pollen from the closed anthers, which were covered by the tepals, or any other substance, even when observing in detail. These bees seemingly only moved up and down the inflorescence very slowly, possibly in search for pollen.

Spathiphyllum croatii had a broader pollinator spectrum with *Plebeia* sp. and *Apis mellifera* as the most frequent visitors, followed by three less frequent stingless bee species, and males of two euglossine species. The plants grow in dense, large clusters, where many inflorescences of both sexual phases are found at a time. Due to the longer duration of the male flowering phase, there are always more inflorescences in male than in female phase, offering abundant amounts of pollen. Pollen grain numbers per inflorescence and P/O ratios were higher than in other *Spathiphyllum* species (Chouteau et al., 2008; Hentrich et al., 2010). We suppose that the large pollen amounts offered in the *S. croatii* population are an attractive and reliable food resource for the observed pollen-collecting bee species and that they therefore regularly visited the inflorescences in high numbers. Since female phase inflorescences appear much like those of the male phase, the bees probably confused the inflorescences with each other, and also landed on the female phase ones (Montalvo and Ackerman, 1986). However, they left them immediately when not finding any pollen. Apparently, these visits were long enough for successful pollination. Due to the high ratio of male to female phase inflorescences, the probability of landing on a male phase inflorescence at their subsequent visit was always higher, thereby preventing the bees from getting discouraged and assuring the constancy of visits in

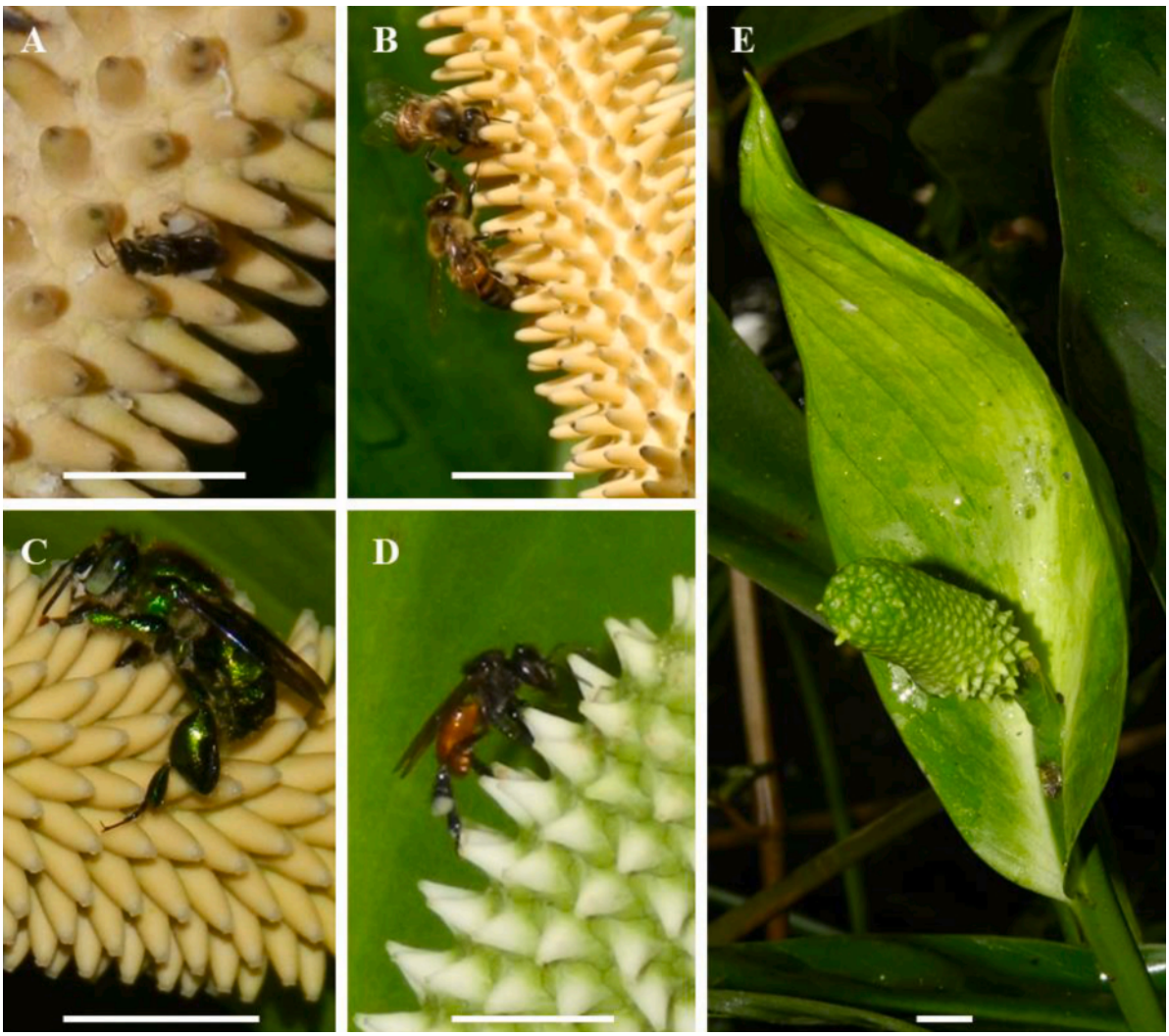

Fig. 5. Flower visitors of the investigated *Spathiphyllum* species: *S. croatii*: A: *Plebeia* sp. collecting pollen of a male phase inflorescence; B: *Apis mellifera* collecting pollen of a male phase inflorescence; C: *Euglossa viridissima* wiping its front legs on a pistil of a female phase inflorescence. D: *Trigona fulviventris* crawling on a female phase inflorescence of *S. ortgiesii*. E: Natural hybrid of *S. croatii* and *S. ortgiesii*. Scale bar = 1 cm.

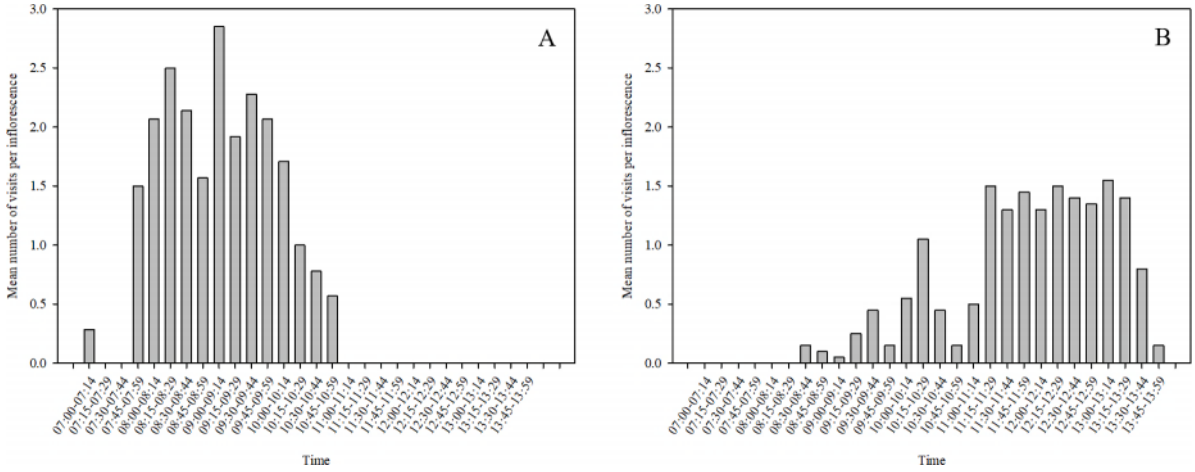

Fig. 6. Mean number of visits to one inflorescence of *Spathiphyllum croatii* (A; $n = 14$ inflorescences; 56 h of observations) and *S. ortgiesii* (B; $n = 20$ infl.; 140 h of observations) in 15 min intervals from all visitor observations conducted in the study at male and female inflorescences.

Table 3
Main floral scent compounds of *Spathiphyllum croatii* and *S. ortgiesii* (compounds represented in > 50% of the samples of a species and with mean relative amounts > 2% of total peak area in any sample). The compounds are grouped according to their chemical class and are presented within each class in the order of their Kovats retention index. The values indicate the median and range (R) of the percentages of total peak area of each compound. N indicates the number of samples in which each compound was found (*F* = female phase, *M* = male phase) from a total of 16 samples (8 female/8 male phase) of *Spathiphyllum croatii* and 12 (8 female/4 male phase) of *S. ortgiesii*. A detailed and complete list of all scent samples and compounds identified for both species is provided in Appendix A and B.

Kovats retention index	Chemical class/compound	*Spathiphyllum croatii*			*Spathiphyllum ortgiesii*		
		Median	R	n	Median	R	n
	Terpenoids						
993	β-Myrcene	0.04	0.02- 0.29	5 (F)	1.31	0.11- 2.20	8 (F)
		0.03	0.02- 0.71	4 (M)	15.93	0.82–57.86	4 (M)
1513	(*E,E*)-α-Farnesene	14.64	0.29–25.38	8 (F)			
		18.61	0.26–71.32	8 (M)			
1626	(*E*)-α-Farnesene epoxide	11.66	6.42–24.88	7 (F)			
		18.86	7.78–44.30	7 (M)			
	Aromatic compounds						
1100	Methyl benzoate	1.09	0.03–35.38	8 (F)			
		5.58	1.01–35.32	7 (M)			
1118	2-Phenylethanol	0.18	0.05- 1.80	8 (F)	93.43	87.42–99.42	8 (F)
		0.37	0.04- 1.50	7 (M)	50.26	48.41–97.71	3 (M)
1203	Methyl salicylate	17.71	1.08–57.63	8 (F)			
		9.22	2.93–13.45	8 (M)			
	Nitrogen-containing compounds						
1143	Phenylacetonitrile	3.96	1.63- 9.59	8 (F)			
		3.13	0.48- 4.25	8 (M)			
	C5-branched chain compounds						
732	Isoamyl alcohol				4.52	0.48–11.18	8 (F)
					33.60	1.46–42.14	4 (M)
	Unknowns						
1255	Unknown 2; *m/z*: 117, 91, 65, 89, 39, 135	2.78	0.46–11.41	8 (F)			
		2.33	0.03–11.06	8 (M)			
1276	Unknown 3; *m/z*: 117, 91, 65, 89, 39, 32	2.42	0.46–8.76	8 (F)			
		1.32	0.05–7.45	8 (M)			

the *Spathiphyllum*-population.

Spathiphyllum friedrichsthalii and the understory palm *Geonoma macrostachys* share growth habit and sex-ratios with *S. croatii* (Montalvo and Ackerman,1986; Olesen and Balslev, 1990; Knudsen, 1999; Knudsen et al., 1999, 2001). Interestingly, the pollinator spectrum of these three species is composed of almost the same insect groups, which suggests that these characteristics might explain the high abundance of stingless bees as pollinators. Montalvo and Ackerman (1986) compared this pollination system with "mistake"-pollination of monoecious or dioecious plants (Baker, 1976; Little, 1983; Dafni, 1984). "Mistake"-pollination may be more frequent in dichogamous tropical understory plants than is known so far.

The only documented male euglossine species in our study were *Euglossa mixta* and *E. viridissima* in *S. croatii*. The first species was observed just once during a very short visit at a male phase inflorescence. Thus, its role as a pollinator is questionable. *Euglossa viridissima* was more frequent, showed a typical scent collecting behavior (Vogel, 1966; Evoy and Jones, 1971; Whitten et al., 1989), and pollinated the flowers. Since the bees were mainly brushing the style, this tissue might be an osmophore (scent emitting tissue), although our SEM analysis did not show a highly increased surface as is typical for osmophores in other plants (Vogel 1963b; Stern et al., 1987). Due to their low frequency as visitors in comparison to the pollen-collecting bees, we consider *E. viridissima* as minor pollinators, despite a potentially high single visit efficiency. Nevertheless, they may play an important role for the genetic diversity of *S. croatii*. Euglossine bees cover large distances in their foraging flights (Janzen, 1971; Williams and Dodson, 1972; Roubik and Hanson, 2004) and might promote pollen flow between scattered populations of *Spathiphyllum* (see also Montalvo and Ackerman, 1986). In contrast, stingless bees are thought to be short-distance flyers (Wille, 1983; Roubik, 1989).

The fact that pollen-collecting bees were dominating the visits to the study plants was an unexpected result, because most studies mention male euglossine bees as most abundant visitors and main pollinators of *Spathiphyllum* species (Williams and Dressler, 1976; Schwerdtfeger et al., 2002; Hentrich et al., 2010). Most major scent compounds of both study species are also found in "perfume flowers" and are known to attract euglossine bees when applied as pure substances (Ackerman, 1989; Gerlach and Schill, 1991; Whitten and Williams, 1992; Eltz et al., 2005; Del Mazo Cancino and Damon, 2007; Hentrich et al., 2010). Therefore, we expected far more male euglossine individuals than observed. Studies of Euglossini in the Los Tuxtlas region show a lower species diversity than in other regions of the Neotropics, but a normal abundance, with *E. viridissima* as one of the most common species (Zimmermann et al., 2011; Martínez-Cervantes, 2019). In comparison to the two *Spathiphyllum* species studied here, *S. friedrichsthalii* was visited by males of 15 euglossine species, besides stingless bees on Barro Colorado Island (BCI), Panama (Montalvo and Ackerman, 1986). The lower number of euglossine bee species as visitors in our study can probably be explained by the fact that Mexico is the northernmost boundary of the natural distribution of Euglossini (Roubik and Hanson, 2004). Therefore, the euglossine species number is generally much lower than in Central or South America (e.g., 44 euglossine species at BCI, Panama; Ackerman, 1989). Finally, the low individual numbers of male euglossine visitors could be attributed to many factors, such as population dynamics, impact of human activity or the unspecific attractiveness of floral scent. Future studies should analyze these issues in more detail and might reveal additional or different euglossine visitors to the flowers of *S. croatii* and *S. ortgiesii* when conducted in the southern regions of Mexico or Honduras.

Each of the *Spathiphyllum* species studied had specific major pollinators that did not overlap with each other. This is remarkable, given that stingless bees are considered true generalists in the selection of the flowers they exploit (Roubik, 1989). So why should the observed bees not visit both plant species, in particular because access to pollen was not restricted by any morphological barrier? Regarding the ecology of the *Spathiphyllum* species studied, there was an outstanding difference in the growth habit and the habitat of the plants. While *S. croatii* builds up large populations at open, sometimes anthropogenically altered sites, *S. ortgiesii* grows scattered in low densities in the understory of preserved

forests. This in turn creates different conditions for their exploitation of flowers and could have had a strong influence on the pollinator spectra of the plants. According to Johnson and Hubbell (1975), *Trigona fulviventris* is assigned to the low-density specialists that visit widely spaced or isolated plants and mainly forage solitary in a spatially dispersed manner. This would explain the presence of *T. fulviventris* as the major pollinator in *S. ortgiesii* and the low frequency of its visits. We have not found any studies that provided information for whether the bee species that visited *S. croatii* were low-density or high-density specialists. However, it is likely that the amount of pollen offered by each small population of *S. ortgiesii* was too small for the profitable exploitation of this resource for other bees or/and that the bees that visited *S. croatii* belong to the high-density specialists (Johnson and Hubbell, 1975).

At the Laguna Azul site, where the habitats of the two *Spathiphyllum* species overlapped, we observed very few (< 1%) visits of *T. fulviventris* to *S. croatii*. Surprisingly, we were able to identify four potential natural hybrids at that site (Fig. 5E), which might be the outcome of pollen transfer from one species to the other by *T. fulviventris* and thus a possible proof of an incomplete reproductive barrier. The hybrids clearly differed in their morphology from their parents so that confusion of species identity can be excluded. Manual pollination experiments showed that both *Spathiphyllum* species studied are compatible with each other and may produce F1 hybrids (P. Díaz Jiménez, unpubl. data). The sharing of *T. fulviventris* as a pollen vector between the two species might therefore lead to introgression. However, such events seem to be rare due to the different habitats in which the plants occur.

Conclusion

Reports about the pollination of the genus *Spathiphyllum* have hitherto given the impression that the vast majority of species are pollinated by scent-collecting male euglossine bees (Williams and Dressler, 1976; Schwerdtfeger et al., 2002; Hentrich et al., 2010). With our study, we present two more cases of *Spathiphyllum* species that are pollinated by pollen-collecting bees, one of which was even pollinated by a single stingless bee species. A broader survey of the reproductive biology of the genus might not only reveal further *Spathiphyllum* species that are pollinated by stingless bees but could also expand the range of pollination systems beyond predominant male euglossine-pollination.

Many other *Spathiphyllum* species are known to share the growth habit of either *S. croatii* or *S. ortgiesii*. Sporadic observations showed that species that grow scattered in the forest frequently produced a very faint floral scent or did not smell at all (at least to the human nose), while species growing in larger aggregations produced an intense scent (Díaz Jiménez et al., 2019a; G. Gerlach, P. Díaz Jiménez pers. obs.). This could be an indication that other *Spathiphyllum* species have a similar pollination biology as the species described in our study, and that the growth habit and/or the habitat in general is linked to the reproductive strategy of these species.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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