# A novel pollination mode, saprocantharophily, in *Duguetia cadaverica* (Annonaceae): A stinkhorn (Phallales) flower mimic

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## ARTICLE INFO

## ABSTRACT

*Duguetia cadaverica* (Annonaceae), a small understory tree of humid primary forest from the Guianas to Pará state, Brazil, unites several unusual blossom and floral characters such as flagelliflory and putrid-smelling flowers, respectively. The few pollination studies conducted in the large genus *Duguetia* have shown that species are usually cantharophilous, pollinated by either small (mostly Nitidulidae) or large specialized dynastid (Scarabaeidae) beetles. Foul-smelling flowers are a novelty within the genus, and to better understand their significance, we undertook a study of the reproductive biology and flower scent chemistry of *D. cadaverica*. In a primary forest of French Guiana, we observed and measured morphology and phenology of trees and flowers; additionally, flower pollination chamber temperature was measured and insect visitors to flowers observed. Flower scent was collected *in situ* and later analyzed in the laboratory by GC–MS. Flowers are visited by small beetles of a single *Pycnocnemus* species (Nitidulidae), which are the only insects observed to enter the pollination chamber. Moreover, flowers evince a rhythm in sexual stage, scent emission and temperature, which finds correspondence in behavioral characters of the putative nitidulid pollinator, such as timing of entry and exit from the pollination chamber. Floral scent analysis revealed an unusual, previously undescribed combination of chemical odor classes. The earthy, rank flower scent contained 18 compounds, among them fatty acid derivatives, terpenoids and N- and S-bearing compounds. The most abundant volatiles were 1-octen-3-ol, 3-octanone, and (*E*)-2-octenol, which are characteristic earthy odors of fungi; additionally, there were sulfides and 4-methylpentanoic acid, which are molecules associated with carcass and cheese odors, respectively.

Saprocantharophily, discovered in *Duguetia cadaverica*, is a novel pollination mode for Annonaceae. The beetle pollinator *Pycnocnemus* sp., which belongs to the *Oxycnemus* genus complex having fungal hosts of the order Phallales, appears to visit the flowers through deception. This extraordinary pollination system, whereby flowers mimic olfactory and visual cues of fungi, is rare and previously only known from three angiosperm families (Araceae, Aristolochiaceae and Orchidaceae) wherein the pollinators were flies.

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## Introduction

The genus *Duguetia* (Annonaceae) comprises 93 species (89 New World tropics, 4 central West Africa) and is the third largest Neotropical genus of the family after *Guatteria* and *Annona* (Maas et al., 2003). Data on the pollination ecology of *Duguetia* are scarce and only nine species have been studied (Webber and Gottsberger, 2003); this said, flower morphology in the genus is relatively homogeneous. From the aforementioned studies, it appears that flowers are usually pollinated by either small (mostly Nitidulidae) or large specialized dynastid (Scarabaeidae) beetles, with the onset of anthesis being diurnal in the former and nocturnal in the latter case. Four phases of anthesis were recognized: (1) attraction of beetles during the female phase; (2) changes from female to male phase; (3) pollen release; (4) collapse of the pollination chamber by dropping of petals and departure of beetles carrying pollen attached by sticky and/or resinous floral exudates.

Flagelliflory, an unusual form of cauliflory wherein flowers are borne on long, whip-like shoots, occurs in three *Duguetia* species: *D. cadaverica*, *D. flagellaris* and *D. sessilis*. Among Neotropical Annonaceae, flagelliflory is known also to occur in four other genera: *Anaxagorea*, *Hornschuchia*, *Stenanona* and *Guatteria* (Maas

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et al., 2003; Erkens et al., 2008). Its presence among these disparate genera is considered to be the result of independent origins (Maas et al., 2003). The basic inflorescence structure in *Duguetia* (and Annonaceae generally) is a cyme. In *D. cadaverica*, the flagellum is an expanded cyme, whereas in *D. flagellaris* and *D. sessilis*, it is a shoot with expanded internodes bearing compact cymes, hence, these two flagella forms are non-homologous (Maas et al., 2003). This difference can also account for the observation of flagella rooting and cloning in *D. flagellaris* (Maas et al., 2003) and its absence in *D. cadaverica*.

*Duguetia cadaverica* Huber is a slender understory treelet (to approx. 3 m tall, 9 cm dbh) that grows in humid sites from the Guianas to the state of Pará, Brazil (Maas et al., 2003). Like its congener, *D. flagellaris*, *D. cadaverica* is a locally common species and bears moderately large crimson and white flowers that have a pollination chamber formed by juxtaposition and reflection of the free petals. However, unlike *D. flagellaris* wherein flower scent is fruity, in *D. cadaverica*, the odor is fetid and this character was the inspiration for the specific epithet "cadaverica". We hypothesize that this distinctive, unpleasant odor is a specialization to attract a specific pollinator or functional pollinator group. To test this hypothesis, we studied flower morphology, phenology, pollen to ovule ratio, insect visitors, as well as floral temperature and scent in a population of *D. cadaverica* at a near pristine site in French Guiana.

## Materials and methods

### Study site

The research was conducted near Crique Nouragues, northeast of the Inselberg camp (4°5′N, 52°41′W) at the Nouragues Natural Reserve in French Guiana. The principal study took place in a population patch of 35 individuals growing a minimum 2 m apart in an area of 1500 m$^2$ (designated P1) in 2004 (late February to early April). A second study was conducted for 9 days in early May 2008, in a 600 m$^2$ plot within P1 (designated P1-1, containing 14 individuals), since part of the original site had been destroyed by an encampment of Foreign Legion recruits.

### Morphology and phenology

Trees were measured (height and dbh) and number, length and position of flagella on each individual was recorded (P1). Additionally, all floral buds on flagella were counted and measured, and their development followed over the ca. 2 month-long observation period in the natural setting with none being bagged (P1). Buds were classified as small (<8 mm), medium (8–15 mm) and large (>15 mm). Measurements taken from six dissected flowers formed the basis for morphological descriptions. Flowers were observed several times per day and several characteristics noted (e.g., flower opening, maturation, insect presence, pollination chamber temperature [only P1-1], scent emission) on flagella of 35 (P1) and 14 individuals (P1-1). Additionally, number of individuals bearing fruit was recorded for P1-1.

### Breeding system

Pollen to ovule (P/O) ratio was determined according to Cruden (1977). Three anthers from each of four flowers were dissected and all pollen grains counted using a cell-counter (Casy®1, Schärfe System, Germany). The number of pollen grains per anther and number of anthers per flower allowed us to estimate total pollen for a whole flower. Ovule number was determined by dissecting four flowers, counting all ovules and then calculating average ovule number.

### Flower temperature

Pollination chamber temperatures were measured for a total of 20 flowers using a manual NiCr-Ni temperature sensor (Therm 2256-1, Ahlborn Mess-und Regeltechnik, Germany). Floral temperature in relation to ambient temperature was checked periodically several times during the day and evening, so that a single flower was the source of several measurements. To measure flower temperature, the sensor was placed onto the basal tissue of the inner petals, as experience showed that this was the site of highest temperatures.

### Flower scent

Volatiles were collected from three female-stage flowers and one flower transitioning from female to male stage (samples of male stage flowers were lost), using a standard dynamic headspace method following Teichert et al. (2009). *In situ*, a single flower was enclosed in an inert oven-bag from which air was drawn for 3 h (150 ml/min) and passed through a glass tube filled with the adsorbents Tenax TA 80/100 (25 mg) and Carbopack B 20/40 (40 mg) using a battery-operated pump. Scent from empty bags was collected to discriminate floral scent from ambient contaminants. The absorbed scent was subsequently eluted from the adsorbents using 0.3 ml of high-grade acetone, and frozen. All samples were analyzed on a Varian Saturn 3800 gas chromatograph (GC) fitted with a 1079 injector and a ZB-5 column (5% phenyl polysiloxane, length 60 m, inner diameter 0.25 mm, film thickness 0.25 μm, Phenomenex), and a Varian Saturn 2000 mass spectrometer (MS). Prior to analysis, 100 μg of nonadecane was added as an internal standard to three of the four samples. One microliter of the acetone-floral scent samples was placed in a quartz vial of the GC injector port by means of a ChromatoProbe kit (Amirav and Dagan, 1997; Dötterl et al., 2009), and analyzed following Dötterl et al. (2005), using Saturn Software package 5.2.1. To identify floral scent compounds from the GC–MS spectra, we used NIST 08 and MassFinder 3 databases; identifications were confirmed by comparison with retention times from published data (Adams, 2007), and in some cases also by comparison of mass spectra and retention times with those of authentic standards.

### Flower visitors

Flower visitors were collected from inside the pollination chamber, examined using a hand lens for the presence of pollen and placed in alcohol for later identification. Voucher specimens are deposited in the collection of the Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia) and in the insect collection of the herbarium ULM. Plant vouchers are deposited at the herbaria CAY and ULM.

## Results

### Morphology and phenology

Measurements at P1 showed that mature trees ($n = 35$) were $3.2 \pm 0.8$ m (mean ± SD) tall and had a dbh of $8.7 \pm 2.7$ cm.

The flagelliflorous inflorescences are perennial structures, which become longer after each successive, sympodially produced flower (Fig. 1). The whip-like flagella turn upward distally suggesting that at flowering bud stage they are more or less negatively geotropic (Fig. 1B–D). When flowers mature directly on the trunk or on aerial flagella, they appear upright (Fig. 1D). When on the ground, leaf litter accumulates over the flagella such that the mature flowers appear to be arising directly from the soil (Fig. 1B). The 35 trees had a total of 201 flagella, which varied from 3 to

**Fig. 1.** Habitat and morphology of *Duguetia cadaverica*. (A) Treelet (ca. 3 m) bearing flagella at base (circumscribed by light box); (B) close-up of 1 A light box showing basal flagella, blue arrows indicate flowers and near mature flower buds, bar = 10 cm; (C) Treelet bearing many flagella higher up on trunk (ca. 1.5 m) – note how apices (floral buds) are negatively geotrophic; (D) mature flower and near mature fruit borne basally – note three white spongy mounds having a median canal, which form a star-like entrance into the pollination chamber (pc), stigmas of gynoecial mound just visible inside pc, bar = 3 cm; (E) immature fruit growing along the ground, blue arrows indicate fruits – note angle of treelet base, bar = 10 cm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

191 cm long (57.4 ± 38.9 cm, mean ± SD) and the average number of flagella/tree was 5.7 (±3.4, SD).

Of 364 initial buds on 201 flagella, 118 aborted (32%). Of the remaining 246 buds, 99 went on to develop into flowers and 147 buds remained at the time of the study end. Of the 99 flowers produced during the observation period, 20 developed into fruits (fecundity = 20.2%). Of these latter, we were able to observe 8 to near maturity. Of the 14 study individuals at P1-1, six individuals bore one (rarely two) fruit, for a total of 7 fruits. The highest number of open flowers on an individual/day was 6, but more commonly 2 or 3 ($\bar{x}$ = 2.5; $n$ = 35) were produced; after producing a flower, there was usually at least a 1-day lag before another flower opened.

The flower bud is formed by 3 fused protective sepals that are brown and resemble leaf litter (Fig. 1B). When in bloom these sepals alternate with 2 whorls of 3 petals each (Figs. 1D and 2A). The reflexed outer-whorl petals are ovate, 2–2.5 cm long and 0.5–1 cm wide and crimson in color. Inner-whorl petals are ovate, reflexed midway, about the same length but generally wider, also crimson-colored, but distinguished by a medial white spongy mound divided into two parts by a central canal. The neighboring appressed half-mounds together form 6 points of a narrow star-like opening (ca. 5 mm wide) that lead to the pollination chamber (Figs. 1D and 2A).

Within the pollination chamber are numerous (91 ± 21) short (ca. 1 mm) stamens. The bright crimson filaments are flanked by white anthers, each of which is crowned by a prolongation of the filament (Fig. 2A). The central gynoecial mound rises above the staminal whorl and bears 18 ± 4.5 free, slender, white carpels (Fig. 2A). The last formed central 3 or 4 carpels appear sterile.

Flowers are protogynous and bloom for about 24 h. They begin to open early in the morning (ca. 05:00) and scent emission is roughly concomitant. At this time, stigmas are shiny with exudate (indicative of receptivity) and anthers indehiscent; thus, flowers are in the functional female stage. Between 16:00 and 17:00 h, stigmas become dry and scent is considerably diminished as well as altered
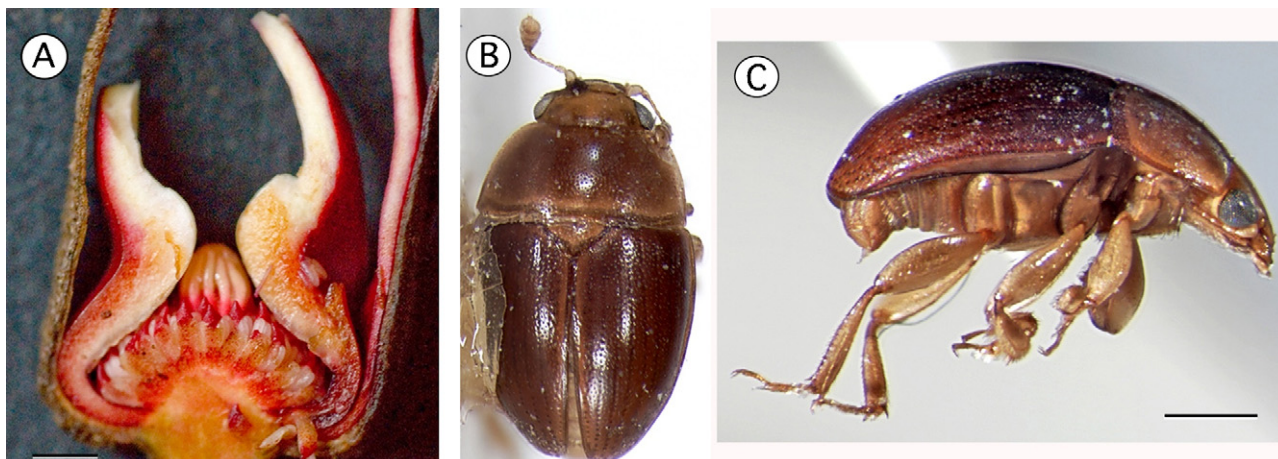
**Fig. 2.** *Duguetia cadaverica* flower and putative pollinator *Pycnocnemus* sp. 1 (Nitidulidae: Cyllodini). (A) Near medial longitudinal section through an opening female-stage flower – note indehiscent white anthers topped by crimson deltoid prolongation of the filament in foreground and glistening white stigmas in background (cut tissue rapidly turns yellow-brown), bar = 2 mm; (B and C) dorsal and lateral view, respectively, of *Pycnocnemus* sp. 1, bar = 0.5 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(see below). Anthers open and pollen is shed, marking the beginning of the functionally male stage. The following morning from 05:00 onwards, the petals and stamens drop (i.e., the pollination chamber collapses), marking the end of anthesis.

### Breeding system

There were $18,396 \pm 1053$ (mean $\pm$ SD) pollen grains and $23 \pm 4$ ovules per flower, respectively, giving a P/O ratio of $799 \pm 273$.

### Flower temperature

We found that temperature differences between the pollination chamber and ambient air reached a maximum of $1.4\,°C$ and $1.3\,°C$ during mid-female stage (midday) and beginning male stage (>16:15), respectively (Table 1). By 07:00 the following morning, flowers, now at the end of anthesis, would be either the same temperature or colder than ambient air (data not shown); in this stage they fall apart when touched.

### Flower scent

The morning of the onset of anthesis, flowers emit a strong unpleasant scent (sometimes noticeable to 1 m distance) reminiscent, to our noses, of very mature Camembert cheese, mushrooms and old damp socks with an additional vomit-like note (female stage). Toward 15:00, the scent begins to diminish and from about 16:00 onwards (male stage), the weak scent loses its most unpleasant aspects but retains a yeasty, mushroomy, Camembert-like fragrance. Out of 18 detected compounds of the odorous substances, 16 were identified. GC–MS analyses revealed that flowers emitted fatty acid derivatives, terpenoids and N- and S-bearing compounds. Among these, fatty acid derivatives dominated the samples, contributing at least 93% to the total amount of scent emitted (Table 2). The bouquet was dominated by 1-octen-3-ol, which represented $59 \pm 18.3\%$ of total volatiles, followed by (*E*)-2-octen-1-ol ($19.4 \pm 12.6\%$) and 3-octanone ($4 \pm 11.8\%$).

### Flower visitors

The sole visitors ($n = 9$) collected in the pollination chamber were small beetles (ca. 1–2 mm) belonging to a new species: *Pycnocnemus* sp., (Nitidulidae: Cyllodini, to be described elsewhere; Fig. 2B and C). These beetles arrived in the morning (before 11:00)

and entered the pollination chamber of female-stage flowers, where they remained until after pollen shedding (male stage), usually the following morning when petals dropped. Beetles caught from female-stage flowers had little or no pollen on them, whereas the single one caught in male-stage flowers had its dorsal surface covered with pollen stuck to it by a sticky substance.

## Discussion

The reproductive biology of *Duguetia cadaverica*, as revealed by the multifaceted floral traits examined here, suggests a mimicry pollination system in which insects are deceived by olfactory and visual cues into visiting the flowers. The flower has a scent associated with both sapromyiophilous plants and stinkhorn fungi (Phallaceae). However, the organization and construction of the flower, and lack of visiting flies, argue against a fly-pollination syndrome but are consistent with a beetle-pollinated one. Moreover, the pollination chamber visitor, a nitidulid beetle, belongs to a group specializing on stinkhorn fungi.

### Scent chemistry

In *Duguetia cadvaerica*, flower scent is characterized chemically by the combination of (in order of decreasing abundance) $C_8$ fungal compounds, 4-methylpentanoic acid and oligosulfides. Although $C_8$ fatty acid derivatives and oligosulfides are sometimes encountered together in angiosperm flower and stinkhorn fruiting body scents (see Johnson and Jürgens, 2010), the combination with 4-methylpentanoic acid is novel. The three $C_8$ fatty acid derivatives 1-octen-3-ol, 3-octanone, and (*E*)-2-octen-1-ol accounted for a minimum of 77% (flower 1, Table 2) and up to nearly 93% (flower 3, Table 2) of the floral scent constituents. These molecules are characteristic fungal volatiles (Fäldt et al., 1999; de Pinho et al., 2008) and associated with the earthy, yeasty scent of mushrooms (Kaminski et al., 1972; Buchbauer et al., 1993). (*E*)-2-Octen-1-ol is found only rarely in flower scent, but 1-octen-3-ol and 3-octanone are reported from floral scents of several diverse plant families (Knudsen et al., 2006). Recently, 1-octen-3-ol was reported from the fruiting body of the fungus *Clathrus archeri*/Clathraceae, Phallales (Johnson and Jürgens, 2010). In most plants, these $C_8$ fatty acid derivatives occur as low-level or trace constituents and it is unclear if at these levels they are always produced by the plants themselves, as there is some evidence to suggest that they may be the

**Table 1**
Temperature in °C of pollination chamber ($T_{pc}$) and ambient air ($T_{aa}$), recorded during different flower sexual stages (F = female, M = male) in *Duguetia cadaverica*, and the resulting temperature difference ($\Delta T$). For each record, $\Delta T$ calculated as $T_{pc} - T_{aa} = \Delta T$.

| Time period (h) | Stage | $T_{pc}$ | | $T_{aa}$ | | $\Delta T$ | | $N$ | |
|---|---|---|---|---|---|---|---|---|---|
| | | Avg $T_{pc}$ | Min $T_{pc}$ – Max $T_{pc}$ | Avg $T_{aa}$ | Min $T_{aa}$ – Max $T_{aa}$ | Avg $\Delta T$ | Min $\Delta T$ – Max $\Delta T$ | Records | Flowers |
| 06:00–08:00 | F | 23.6 | 23.4–23.8 | 23.0 | 22.6–23.3 | 0.5 | 0.4–0.9 | 7 | 4 |
| 11:00–13:00 | F | 25.0 | 24.4–25.9 | 24.5 | 23.9–25.4 | 0.5 | 0.1–1.4 | 16 | 11 |
| 15:00–16:15 | F–M | 25.5 | 24.5–26.6 | 25.2 | 23.6–26.4 | 0.3 | −0.2 to 0.9 | 10 | 7 |
| 16:30–17:30 | M | 26.5 | 26.0–26.8 | 25.7 | 25.3–26.7 | 0.7 | −0.1 to 1.3 | 4 | 4 |

product of fungal endophytes in flowers and/or inflorescences (S. Dötterl, unpubl. data). Furthermore, their role (if any) in pollinator attraction remains to be determined. However, in the rare cases when 1-octen-3-ol and 3-octanone, or other mushroom-like compounds, occur as major constituents of floral scent, they are found in flowers that typically mimic food and brood sites of fungivorous flies (Johnson and Jürgens, 2010; Dobson, 2006; Kaiser, 2006; see also below) or that are pollinated by bats (Knudsen and Tollsten, 1995).

Isocaproic acid (4-methylpentanoic acid), which occurred in three of four samples (Table 2), is known from several orders of fungi (Pyysalo, 1976; so far not in Phallales), but has been reported only once from floral scents (Knudsen et al., 2006) in an analysis of inflorescence odors of *Amorphophallus* and *Pseudodracontium* (Araceae) by Kite and Hetterscheid (1997). These authors found isocaproic acid in a single species, *Amorphophallus elatus*, where it was the sole compound emitted by the strong cheesy-scented inflorescences (Kite and Hetterscheid, 1997). Analysis of additional *Amorphophallus* species has revealed a similar pattern in five more species (G. Kite, pers. comm.). Where studied, *Amorphophallus* species have been found to be pollinated either by carrion beetles or nitidulids (e.g., van der Pijl, 1937; Sivadasan and Sabu, 1989; Beath, 1996; Punekar

and Kumaran, 2010). However, the pollinators of species emitting isocaproic acid are unknown (Wilbert Hetterscheid, pers. comm.).

The oligosulfides (dimethyl trisulfide and dimethyl tetrasulfide, two and one sample(s), respectively, Table 2), occurred at much lower concentrations (<5%) and are known to produce characteristic nauseating odors, like those released from liquid petroleum, and of rotting meat or cadavers (Kite et al., 1998). Even very small amounts of these compounds are noticeable and dimethyl trisulfide is detectable to humans in concentrations as low as 6.2 mg m$^{-3}$ (Ruth, 1986). Oligosulfides are found in scents of flowers belonging to diverse plant lineages, and are frequently associated with saprophilous (often Araceae) and certain chiropterophilous plants (Borg-Karlson et al., 1994; Knudsen and Tollsten, 1995; Kite and Hetterscheid, 1997; Stránský and Valterová, 1999; Jürgens et al., 2006). They have also been reported as volatiles of the sexual bodies of two stinkhorn fungi (Phallales), *Phallus impudicus* (Borg-Karlson et al., 1994) and *Clathrus archeri* (Johnson and Jürgens, 2010). In both cited studies, the fetid scents of the respective stinkhorns were compared with similar smelling angiosperm blossoms (flowers – Johnson and Jürgens, 2010; inflorescences – Borg-Karlson et al., 1994). In both papers a similar conclusion was drawn, that chemical similarity between reproductive structures' scent of the stinkhorn

**Table 2**
Chemical composition of the floral scent of four *Duguetia cadaverica* flowers, given as total amount followed by percentage amount of each compound. RI = Kovats retention index. Values in bold represent compounds present at >5.0%. tr = trace amount, here given as <0.05% of total. nd = not determined. Unknowns were pooled and the number of pooled compounds is indicated by the superscript digits.

| | RI | Flower 1 | Flower 2 | Flower 3 | Flower 4 |
|---|---|---|---|---|---|
| Time of scent collection (h) | | 08:05–10:35 | 10:00–13:00 | 11:35–13:45 | 15:40–18:10 |
| Total amount of scent in sample (μg) | | 11.1 | nd | 5.5 | 3.5 |
| **Fatty acid derivatives** | | | | | |
| 4-Methylpentanoic acid[a] | 945 | 0.5 | **16.7** | 2.6 | – |
| 1-Octen-3-ol[a] | 982 | **47.4** | **39.4** | **74.9** | **74.4** |
| 3-Octanone[a] | 989 | **26.5** | 0.3 | **8.6** | 3.1 |
| Octanal[a] | 1006 | 3.3 | tr | 0.4 | 0.1 |
| (*E*)-2-Octenal[a] | 1065 | 3.0 | 0.6 | 0.5 | 0.5 |
| (*E*)-2-Octen-1-ol[a] | 1071 | **16.7** | **37.7** | **9.3** | **14.0** |
| 1-Nonen-3-ol | 1082 | tr | – | 0.2 | 0.1 |
| 3-Nonanone | 1090 | tr | – | tr | tr |
| Unknowns | | 2.2[8] | 0.8[8] | 0.9[4] | 0.4[3] |
| **Monoterpenoids** | | | | | |
| Linalool[a] | 1103 | tr | – | 0.1 | – |
| **Sesquiterpenoids** | | | | | |
| (*E*)-β-Caryophyllene[a] | 1447 | – | – | tr | – |
| (*E*)-β-Farnesene[a] | 1464 | – | – | 0.4 | – |
| (*E,E*)-α-Farnesene[a] | 1512 | – | – | 0.3 | – |
| Unknowns | | tr[1] | – | 1.1[6] | **5.7[7]** |
| **N-bearing compounds** | | | | | |
| Benzyl nitrile[a] | 1151 | 0.2 | – | 0.2 | 0.9 |
| 2-Phenylnitroethane | 1314 | 0.1 | tr | 0.3 | 0.8 |
| **S-bearing compounds** | | | | | |
| Dimethyl trisulfide[a] | 982 | – | 4.4 | 0.2 | – |
| Dimethyl tetrasulfide[a] | 1235 | – | 0.2 | – | – |

[a] Compounds were identified by comparing mass spectra and retention times with those of synthetic standards.

fungus and angiosperms probably results from convergent evolution to attract sarco- or sapro-philous insects.

### Beetles and hosts

The observation of tiny nitidulid beetles *(Pycnocnemus* sp. 1) being the sole visitors to the pollination chamber of *Duguetia cadaverica* indicates a specialist pollination system. It is important to note that one reason more beetles were found in female stage flowers is because this stage lasts most of the day, and as the insects are very small, this is the stage when we were most likely to see them in the penumbra of the understory. Moreover, the rare but regular presence of a single or few beetle species is a phenomenon characteristic of many cantharophilous flowers (Gottsberger, unpubl. data). The diet of sap beetles (Nitidulidae), so-named because some members feed on the "sap" of tree wounds, is diverse, but commonly they are mycetophagous, saprophagous or anthophagous (Parsons, 1943). The genus *Pycnocnemus* belongs to tribe Cyllodini, more specifically to the *Oxycnemus* complex (Leschen, 1999; Kirejtshuk, 2008). Many Cyllodines are associated with large-bodied Basidiomycota hosts (Leschen, 1999), on which the beetles feed and reproduce. Larval development time is particularly short in Nitidulidae and in tropical cyllodines it might be as short as 2–3 days (Kirejtshuk, unpubl. data). Some cyllodines are fungal generalists, however, several members of the *Oxycnemus* complex are specialist feeders on stinkhorns (Phallaceae, Phallales, Basidiomycota).

Stinkhorns, so-called because of the nauseating odor that accompanies presentation of the gleba and its phallus-shaped receptacle, attract flies, which land and then feed on the deliquescing gleba and its sticky spore masses; the spores pass undigested and are disseminated (Tuno, 1998). Other insects, such as beetles (e.g., Smith, 1956) and bees (Shaw and Roberts, 2002), are also attracted to the fragrant sexual bodies.

Leschen (1999) suggested that some Neotropical members of the *Oxycnemus* complex may be attracted to volatiles that are produced by the fruiting bodies (which typically last 1–3 days) prior to those responsible for attracting flies and in this way, arrive early enough to allow larval development in the fruiting bodies. In a study of insect interactions with the stinkhorns *Blumenavia rhacodes* and *Lysurus periphragmoides* in Argentina, Nouhra and de Toledo (1994) reported that *Oxycnemus aterrimus* (sister genus to *Pycnocnemus*) attracted to the odoriferous gleba, fed on it then the degrading receptacle. Gut contents of *O. aterrimus* revealed angiosperm vessels and *B. rhacodes* spores, viability of spores was 36% (Nouhra and de Toledo, 1994). Additionally, beetles were found to carry spores on their bodies and the authors conclude that the beetles are spore dispersers for the two stinkhorns (Nouhra and de Toledo, 1994).

Aside from capturing one beetle covered with pollen from the pollination chamber of *Duguetia cadaverica*, we have nearly no data on the life-cycle of the *Pycnocnemus* sp. However, by analogy with known interactions in their stinkhorn hosts, it seems likely that the beetles ingest stigmatic exudates (as this is a liquid and presumably readily assimilated) and *possibly* pollen and other tissues. Moreover, they may possibly oviposit in the flowers. When in the petals, larvae may develop in the decomposing tissue on the forest floor. With respect to evolutionary trends generally within sap beetles (Nitidulidae), it appears that many groups, as well as other Cucujoid families (i.e., members of the superfamily Cucujoidea), are at an early stage of settling down to a lifestyle involving larval development in decaying flowers (larval and imaginal anthophagy), and these groups demonstrate a regular association with decomposing flowers (Kirejtshuk, 1994). It may be that this substrate is similar enough to fungi so that the shift from mycophagy to saproflorivory is relatively easy.

### Pollination and breeding system

In Annonaceae, cantharophily is almost always associated with a pollination chamber, and this strongly scented structure shelters the flower-visiting beetles (Gottsberger, 1999). In *Duguetia cadaverica*, the pollination chamber temperature can increase up to 1.4 °C above ambient temperatures (Table 1). The warm temperature of the pollination chamber is likely related to odor volatilization (with which it is coincident) but may also be a pollinator "reward" (Seymour et al., 2003). However, studies of heat rewards to beetles by flowers have involved large-bodied scarabs (Dynastinae) and nothing is known with respect to beetles on the order of 1–2 mm long.

We found that the P/O ratio was $799 \pm 273$ (mean $\pm$ SD), which is suggestive of a xenogamic breeding system (Cruden, 1977). Paradoxically, most studied Annonaceae species have been found to be self-compatible (Gottsberger et al., 1980). However, this is offset by the fact that most co-sexual Annonaceae, among them *D. cadaverica*, are strongly dichogamous (usually protogynous) such that the female and male phases do not overlap. Dichogamous systems are generally considered a strategy to insure out-crossing (Lloyd and Webb, 1986).

*Duguetia cadaverica* can form large populations although this species is often not recorded in inventories because its dbh is usually less than 10 cm, a widely used inventory standard. Within a population, flowering is synchronized locally among adjacent subsets and individuals more or less flower alternately among neighbors. The short distances between flowering individuals and limited penetrating power (as determined by human olfactory systems) of flower odor suggest that the beetles migrate from one part of the population to another following the trail of provided cues.

Flagelliflory is possibly best understood as a condition wherein an inflorescence is spatially and temporally extended or drawn out. The advantages of this rare form of flower presentation are not understood, but it is clear that positional effects play a particularly important role as flowers can be on the trunk, in the air or on the ground. We saw only one fruit in the air and collected beetles only from flowers on the ground or near the base of the tree. *Pycnocnemus* sp. 1 can fly, so that reaching flowers presented higher on the tree does not pose any particular problems. Nonetheless, we expect that a certain minimum of open flowers in the vicinity is probably necessary to attract beetles. It might be that flowers farther from the ground serve more an attractive function, adding to local flower density (especially contributing to scent), but are less likely to be pollinated than those nearer the ground. It appears that there is considerable flower bud mortality (32%) and flower fecundity is 20.2%. *Duguetia cadaverica* bears $23 \pm 4$ fertile carpels (mean $\pm$ SD). As each carpel contains a solitary ovule (Maas et al., 2003), there are roughly 20 seeds per fruit. Our survey of 14 individuals (P1-1) revealed that a little less than half of them (6) bore one or rarely two fruit (in all, seven fruits, the products of the previous flowering episode). Calculations suggest that roughly a total of 140 seeds were produced by 6 individuals in a contiguous population patch of 14 individuals. Taken together, our meager data suggests that this is not a particularly efficient reproductive system. However, the known existence of large populations of *Duguetia cadaverica* implies that it is sufficient, with the caveat that the habitat is pristine forest. It is evident that these slender trees, which frequently flower and fruit on the ground, are exceptionally vulnerable to even minor habitat change.

### Fungus mimicry in flowers

We interpret the flower of *Duguetia cadaverica* to be a Phallales fungus mimic. This extraordinary system is supported by our findings on the unusual combination of the floral scent, as well as by the

morphological floral traits and spatial presentation of flowers in the forest habitats of *D. cadaverica*. The well-developed spongy tissue at the entry of the pollination chamber is reminiscent of fungal bodies, and the crimson and white petal coloration might correspond to the fruiting body of *Phallus indusiatus* var. *rosea* (Phallales) recorded from South America (Wright, 1960; Cheype, 2010). Furthermore, the ephemeral flowers are usually presented on or near the forest floor, where the fruiting body of stinkhorns, would normally occur.

Saprocantharophily, mentioned in passing by Burgess et al. (2004; and mistakenly attributed to Bernhardt, 2000), is the beetle equivalent of the better known pollination syndrome sapromyiophily (Faegri and van der Pijl, 1979). We use these terms in the broad sense of pollination by beetles or flies, respectively, that breed or feed on dung, decaying matter or fungi, as it is not yet possible to distinguish more finely between relationships with these substrates (see Jürgens et al., 2006). Although usually subsumed under cantharophily or even sapromyiophily, saprocantharophily is a distinct syndrome rooted in beetle behavioral and morphological characteristics; it is found in several disparate families and genera, e.g., many Araceae, especially *Amorphophallus* (Sivadasan and Sabu, 1989; Beath, 1996; Punekar and Kumaran, 2010) as well as the root holoparasite, *Hydnora africana* (Hydnoraceae; Bolin et al., 2009). However, flowers that mimic the fruiting bodies of fungi are especially rare, and, until now, known from only three families, Araceae, Aristolochiaceae and Orchidaceae (Kaiser, 2006). In these deceptive systems, flies, mainly belonging to the families Mycetophilidae, Sciaridae and Drosophilidae, are attracted to flowers mimicking olfactory and visual cues characteristic of fungi where the insects normally meet and females oviposit (Dobson, 2006; Endara et al., 2010; Lunau, 2002). Among fungus mimics, conclusive floral scent analyses are only available in *Dracula chestertonii*. The fragrance corresponds to a "mushroomy" scent and the $C_8$ fatty acid derivatives 1-octen-3-ol, 1-octen-3-one and 3-octanone make up more than 70% of constituents (Kaiser, 2006). The preponderance of these $C_8$ fungal compounds is similar to what we have found in *Duguetia cadaverica*, however, in contrast to our study species, *Dracula* flowers do not additionally emit S-bearing compounds nor any fatty acids.

Unlike all previously known fungus-mimicking flowers, the flowers of *Duguetia cadaverica* are not trap-blossoms, a fact that might relate to the pollinators being beetles and the construction and organization of Annonaceae flowers. Within the genus *Duguetia*, this pollination system is most comparable to *D. flagellaris*, wherein crimson and white erect flowers are similarly presented on flagella, the sexual phase lasts 24 h, and there is weak floral warming. The two species differ in that the flowers of *D. flagellaris* produce a fruity scent, have initial functioning of female-stage flowers in the afternoon, and likely pollinators are *Colopterus* sp. (Nitidulidae: Cillaeinae) and Thysanoptera (Webber and Gottsberger, 2003).

This is the first reported case of flowers mimicking fungi in Annonaceae and would seem to be an isolated case within the genus and family. Key features of this saprocantharophilous system are the stinkhorn-like scent composition of the flower, protogyny, pollination chamber having a slightly warmer temperature compared to ambient air, flower texture and coloration, ephemeral flower presentation on or near the forest floor and proximity of flowering cohorts. Future studies should be directed at understanding the life-cycle and behavior of *Pycnocnemus* sp., identifying the stinkhorn model and analyzing its scent chemistry.

## Acknowledgments

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