



A perfume-collecting male oil bee? Evidences of a novel pollination system involving *Anthurium acutifolium* (Araceae) and *Paratetrapedia chocoensis* (Apidae, Tapinotaspidini)[☆]



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ABSTRACT

It has been known since Stefan Vogel's observations in 1969 that solitary female oil bees collect fatty floral oils from specialized oil-secreting plants with the aid of hairy patches on either their legs or abdomen, a reward used as food for their larvae and/or to line their brood cells. Similar adaptations are also known from male oil bees, although the purpose of their oil-collecting behavior has not yet been clarified. Here, we describe a novel pollination system involving male *Paratetrapedia* oil bees and the tropical herb *Anthurium acutifolium*. We present ultrastructural morphological details of bee and plant structures involved in this interaction and the composition of floral scents likely mediating pollinator attraction. Inflorescences of *A. acutifolium* were visited almost exclusively by male *P. chocoensis* oil bees. The bees mopped with a hairy patch of their abdominal sterna 3 across the inflorescence surface. During this activity on both staminate and pistillate stage inflorescences, bees' abdomens and legs became loaded with pollen and contacted receptive stigmas. In contrast to what has been observed in other angiosperms visited for the collection of fatty floral oils, the inflorescences/flowers of *A. acutifolium* do not have structures specialized in oil secretion, i.e., elaiophores. These inflorescences, nonetheless, were strongly scented during the time interval they were visited by the bees. Gas chromatography/mass spectrometry (GC/MS) analyses of dynamic headspace floral samples revealed that inflorescences of both anthetic phases emitted scent bouquets consisting mainly of aliphatic esters, indole and uncommon terpenoids (megastigmanes). Interestingly enough, our data suggest that the unusual floral scent of *A. acutifolium* is a perfume reward collected by male *P. chocoensis* oil bees. This pollination system thus bears a remarkable resemblance with the interactions between perfume-collecting male euglossine bees and their preferred flowers, discovered by Stefan Vogel half a century ago.

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1. Introduction

In mutualistic interactions involving angiosperms and pollinating insects, nectar is the most common liquid reward. Some flowers, however, offer rewards other than nectar to visitors (Endress, 1996; Simpson and Neff, 1981), among which are fatty oils and fragrances. Pollination systems involving oil and perfume collection

were both discovered in pioneer research investigations of Stefan Vogel (Vogel, 1966, 1969, 1974).

Fatty oil-secreting flowers do so through trichomal or epithelial elaiophores (Vogel 1969, 1974, 1990). They occur across eleven plant families around the world, being more widely acknowledged in selected genera of Iridaceae, Krameriaceae, Malpighiaceae, Orchidaceae and Scrophulariaceae (Buchmann, 1987; Cocucci and Vogel, 2001; Renner and Schaefer, 2010). The only known pollinators dedicated to the collection of floral fatty oils are female oil bees (Apidae and Melittidae), which use specialized hairy structures on their legs and/or abdomens to gather this resource (Cocucci et al., 2000; Roig-Alsina, 1997; Vogel, 1969, 1974, 1990). Fatty floral oils, together with pollen from the same or different plant species, are mainly used for brood cell provisioning but, sometimes, they

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are also applied in nest construction (Buchmann, 1987; and see references in Schäffler and Dötterl, 2011).

Floral fragrance rewards, produced in osmophores (Vogel, 1963b) of flowers belonging to at least eleven families of angiosperms (Cameron, 2004; Dodson et al., 1969; Dressler, 1982; Roubik and Hanson, 2004; Sazima et al., 1993; Teichert et al., 2009; Vogel, 1963a, 1966) are sought and collected by Neotropical male euglossine bees (Apidae, Euglossini; Eltz et al., 1999; Vogel, 1966). The sole exception are some tephritid flies, which “feed” on volatiles from orchid flower surfaces and accumulate these volatiles inside their rectal glands (Tan and Nishida, 2000). Male euglossine bees collect volatiles with hairy brushes on their fore tarsi and store them in highly complex organs in their hind tibiae (Eltz et al., 1999; Vogel, 1966). The most well-known fragrance sources for these bees are heavily scented flowers of more than 700 species of orchids (Roubik and Hanson, 2004), followed by a number of aroid species (Araceae) belonging to *Anthurium* Schott and *Spathiphyllum* Schott (Croat, 1980; Hentrich et al., 2010; Montalvo and Ackerman, 1986; Schwerdtfeger et al., 2002; Vogel, 1963a,b, 1966). When collecting fragrances from the spadix of an aroid inflorescence (spike like reproductive organ, covered with densely packed hermaphroditic protogynous flowers), the male euglossine bees touch receptive stigmas (during the pistillate phase of anthesis) and anthers/pollen (during the staminate phase of anthesis), thus, ensuring effective pollination (Hentrich et al., 2010).

Several other pollinators have been described among the more than 950 currently known species of *Anthurium* (Boyce and Croat, 2016), including flower weevils (Curculionidae; Franz, 2007), fruit flies (Drosophilidae), gall midges (Cecidiomyidae; Schwerdtfeger et al., 2002), and hummingbirds (Krämer and Schmitt, 1999). Given the low number of species investigated with respect to their pollination ecology within this highly diverse genus, it is only expected that several still unknown pollination systems are yet to be described. In preliminary observations of *Anthurium acutifolium* Engl. (Araceae) at the “La Gamba Field Station” in Costa Rica, we observed that inflorescences are frequently visited by male *Paratetrapedia chocoensis* Aguiar and Melo (2011) oil bees. Although flower visits by male oil bees have not yet been investigated in detail, already Stefan Vogel (1974) mentioned the phenomenon, which was just recently confirmed by Cappellari et al. (2012). It is also known that male oil bees belonging to several species of Neotropical Tapinotaspidini and Tetrapediini exhibit hairy structures similar to those typically found on females, to also collect and store fatty floral oils (Aguiar and Melo, 2011; Cappellari et al., 2012; Vogel, 1990). The biological/ecological function of oil collecting behavior in male oil bees is unknown so far.

Here, we investigate the visitation of *A. acutifolium* inflorescences by male *Paratetrapedia chocoensis* oil bees and address the following questions: I) Are male *P. chocoensis* oil bees effective pollinators of *A. acutifolium* and are its inflorescences associated to other species of floral visitors? II) Do inflorescences of *A. acutifolium* possess trichomal or epithelial elaiophores for the production/secretion of floral fatty oils and if, are these floral fatty oils collected by male oil bees through specialized body structures? III) Do the inflorescences emit detectable floral scents during anthesis and what is the identity of the released volatile organic compounds?

2. Material and methods

2.1. Study species, study time, and study site

Anthurium acutifolium is known from Costa Rica and Panama, where it occurs in tropical and premontane wet forests from sea level to 900 m asl (Croat, 1983). It is a terrestrial, rarely epi-

phytic herb with erect elliptically leaves, erect inflorescences and thick roots (for detailed information see also <http://www.aroid.org/genera/anthurium/pachyneurinum/acutifa.php>), locally often used as an ornamental. Our study was carried out at the “La Gamba Field Station”, Pacific coast of Costa Rica (8°42'46" N, 83°12'90" W; 120 m asl), during the dry season, between January – April of 2015 and 2016.

2.2. Floral anthesis and visitors

Anthesis was studied on five inflorescences from two individuals in 2015 and six inflorescences from four individuals in 2016 (including the two observed in 2015). We conducted sporadic observations (in total ca. 12 h) during the morning (6:00–12:00), in the afternoon (13:00–18:00), and at nighttime (18:00–24:00) for a total of 75 days to check for insect visitors, as well as scent production perceivable to the human nose. Additionally, on five different occasions we placed video cameras (Sony HDR-PJ780) in front of inflorescences and obtained several hours worth of uninterrupted recordings in the morning and afternoon to check for visitation patterns, visiting species and pollinator behavior. On three different occasions in February 2015 we constantly observed inflorescences at the pistillate ($n = 2$) and staminate ($n = 1$) phases of anthesis from 9:00 to 11:00 to record the presence and number of visiting insects in 5 min intervals. The duration of single visits was also recorded ($n = 28$). To find out if individual male oil bees repeatedly visited inflorescences at different days, ten individuals of *P. chocoensis* bees were caught during visitation on *A. acutifolium*, marked with atoxic red paint on the thorax, and subsequently released.

Vouchers of the insect species are deposited at the Entomological Collection of Universidade de Brasilia, Brazil and at the private insect collection of FE at the University of Vienna, Austria.

2.3. Morphological analyses of male oil bees and inflorescences

Nine male individuals of *P. chocoensis* were collected from inflorescences of *A. acutifolium* for microscopic and ultrastructural investigation (stereo microscope Leica-EZ4; digital 3D microscope Leica-DVM6, Leica Microsystems AG, Heerbrugg, Schweiz; scanning electron microscope JSM-6390, Joel, Peabody, USA).

Three individuals immediately frozen after collection were mounted for observation under scanning electron microscopy (SEM); additionally, two other individuals were sequentially washed in different solvents prior to mounting (ethanol 96%, hexane p.a. and acetone 99.8%; all from Sigma-Aldrich, Missouri, USA). In both cases, individuals were dorsally mounted on the thorax, critical point dried (Autosamdri-815, Tousimis, Rockville, USA) and sputter coated with gold (SCD 050, BAL-TEC Maschinenbau AG, Pfäffikon, Schweiz) before scanning.

To check for possible trichomal or epithelial elaiophores involved in fatty oil secretion, as well as for nectar secretion, spadices during the pistillate and staminate phases were photographed in the field under macro magnification (Canon macro lens EF-100 mm on a Canon 60d camera equipped with a ring light Canon MR-14EX) and examined with a hand magnifying glass (10×). SEM images were obtained from one pistillate phase spadix cut and placed in 70% ethanol. Longitudinal and transverse slices were serially dehydrated in ethanol (85% and 96%) and acetone (99.8%) (all from Sigma-Aldrich, Missouri, USA) prior to critical point drying and gold sputtering (see above).

2.4. Scent collection and analyses

Scent samples from four different inflorescences from 3 individuals of *A. acutifolium* were collected using dynamic headspace

methods (see Dötterl et al., 2005; Etl et al., 2016) during the period of strongest scent emission as determined by the human nose and indicated by the presence of visiting male oil bees (9:00–11:00). One individual was sampled during both the pistillate (five samples from one inflorescence) and staminate (two samples from the same and three samples from another inflorescence) phases, one individual during the pistillate phase only (two samples from one inflorescence), and another one during the staminate phase only (four samples from one inflorescence). For scent collection, a rotary vane vacuum pump (G12/01EB, Gardner Denver, Germany) was used with an air flow set at 200 ml/min. Inflorescences were bagged before scent collection with polyethylene oven bags (10 × 20–35 cm; Toppits, Germany) and scent was trapped for 2 min (following an accumulation period of 0–10 min; n = 12 samples) on adsorbent tubes (quartz glass tube: length 25 mm; inner diam 2 mm) filled with 1.5 mg of Carbotrap B (mesh 20–40, Supelco, Germany) and 1.5 mg of Tenax TA (mesh 60–80; Supelco, Germany). In some cases, scent was collected for longer periods of 5–30 min (n = 4 samples), following an accumulation time of 0–15 min. To obtain negative control samples, scent was collected for 2 min from empty oven bags (n = 3).

Samples were analysed on a GC/MS (QP2010Ultra, Shimadzu Corporation, Japan) coupled to a thermal desorption unit (TD-20, Shimadzu, Japan) and equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; 60 m long, inner diameter 0.25 mm, film thickness 0.25 µm, Phenomenex, USA), the same as described by Mitchell et al. (2015). Samples were run at a column flow (carrier gas: helium) of 1.5 ml/min. GC oven temperature started at 40 °C, then was increased by 6 °C per min to 250 °C, and held for 1 min. The MS interface worked at 260 °C, and the ion source at 200 °C. Mass spectra were taken at 70 eV (in EI mode) from *m/z* 30 to 350. The GC/MS data were processed using the GCMSolution Version 4.11 (Shimadzu Corporation, Japan). Compounds were identified by the NIST 11, Wiley 9, FFNSC 2, Essential Oils and Adams 2007 mass spectral databases, and whenever possible confirmed by comparison of mass spectra and retention times with those of authentic standards (available in the stock collection of Stefan Dötterl, University of Salzburg). To determine the amount of scent trapped from an inflorescence, known amounts of monoterpenes, aliphatics, and aromatics were injected into the GC/MS system; mean peak areas of these compounds were used to determine the total amount of scent (see Dötterl et al., 2005).

3. Results

3.1. Anthesis and floral morphology

Inflorescences of *Anthurium acutifolium* consisted of a yellow-greenish spadix, enclosed only during early development by a green spathe. Flowers are hermaphroditic, protogynous, and densely packed around the spadix. No oil-secreting structures (trichome or epithelial elaiophores) are present, and neither were oil droplets or nectar droplets observed on the surface of the tepals, gynoecea or anthers (Fig. 1B and E; Supplementary Figs. 1 and 2).

The pistillate phase (indicated by moist protruding stigmata) lasted for approximately four days, followed by a staminate phase (indicated by consecutively emerging and opening anthers) which lasted for up to two weeks. The two sexual phases never overlapped.

3.2. Floral visitors

In both observation years, the only insects visiting the spadices during both the pistillate and the staminate phases were male *Paratetrapedia chocoensis* oil bees (Fig. 1). A single visit from a

pollen collecting female stingless bee (*Trigona fulviventris* Guérin-Méneville, 1845) to a staminate phase spadix was observed in 2015.

Male oil bees visited the inflorescences between 8:30 and 12:00, when floral scent emission was perceivable. Up to five bees could be observed simultaneously on the spadix of a given inflorescence (Fig. 1F; Supplementary Video 1). The elapsed time during a visit largely differed between individuals, lasting from a few seconds up to several minutes (mean: 3 min 47 s, Min – Max: 2 s – 15 min, n = 28). Visits were interrupted by short hovering flights in front of the spadix (Fig. 1A; Supplementary Video 1). Three marked individuals visited the same inflorescence on the following day.

The bees started their visits typically at the base of a spadix (Supplementary Video 1). Upon landing, they pressed sternum 3 of their abdomens on the surface of the spadix (Fig. 1B, D, E) and slowly walked towards the apex before turning around and walking back to the base of the spadix (Supplementary Video 3). This sequence was repeated until they abandoned the inflorescence. During visits, the bees constantly contacted the surface of the spadix with both antennae alternating (Fig. 1B; Supplementary Video 3).

We found heavy loads of pollen grains on the abdomens and legs of male *P. chocoensis* oil bees when they visited inflorescences of *A. acutifolium* during the staminate phase (Fig. 1C and D; Fig. 3D, E, F; Supplementary Video 2). When visiting pistillate phase inflorescences, bees touched the stigmata with their abdomens, legs and antennae (Fig. 1B; Supplementary Video 2, 3). The bees transferred pollen between spadices of the same plant or different individuals. In few instances, single bees were found switching from staminate to pistillate phase inflorescences (Supplementary Video 2).

Sternum 3 contains a hairy brush (width: 1 mm, length: 0.5 mm), which covers nearly the whole sternum (Fig. 1A, B, C; Fig. 2A). It is comprised of thousands of 10 µm thick and 100 µm long, club shaped and stiff hairs. Each hair contains numerous, short and upwards pointing branches which are very dense at their tips, forming small brushes themselves (Fig. 1E and G; Fig. 2B, D, F). Because the surface of each hair was covered with a fine oily film that made them pack together, details of their ultrastructure (Fig. 2A, C, E; Fig. 3F) were only visible after solvent wash (see material and methods).

Video analyses revealed that male *P. chocoensis* oil bees use the inner serrate-shaped spurs (0.5 mm in length; Fig. 3A and B) of their hind tibia to brush over sternum 3. Although the purpose for such behavior is yet unknown, the 10 µm-spaced teeth in these spurs (Fig. 3C) are a perfect fit for the hairs (Fig. 2B, D, F). One bee was observed repeating the brushing behavior nine times in posterior direction, while being on the spadix of an inflorescence of *A. acutifolium* (Supplementary Video 4). Another bee rubbed its hind legs (basitarsi and likely tibial spurs) on one another during flight (Supplementary Video 5). We observed that the plumose metatarsi of yet another bee were soaked with a shiny liquid (Supplementary Fig. 3).

3.3. Floral scent

Pistillate and staminate phase inflorescences of *A. acutifolium* emitted a clearly perceivable scent in the morning hours during the whole period of anthesis.

Scent emission (as determined by the human nose) was strongest between 09:00 and 11:30, but also noticeable 30 min before and after that time span. The total amount of scent trapped per inflorescence was 459 (45–935) ng/h (mean, Min–Max; n = 7 samples from 2 individuals) for the pistillate phase of anthesis and 1077 (672–2482) ng/h (n = 9 samples from 2 individuals) for the staminate phase (Table 1).

The scent bouquet contained overall 33 volatile compounds, among them aliphatic compounds, terpenoids and a nitrogen

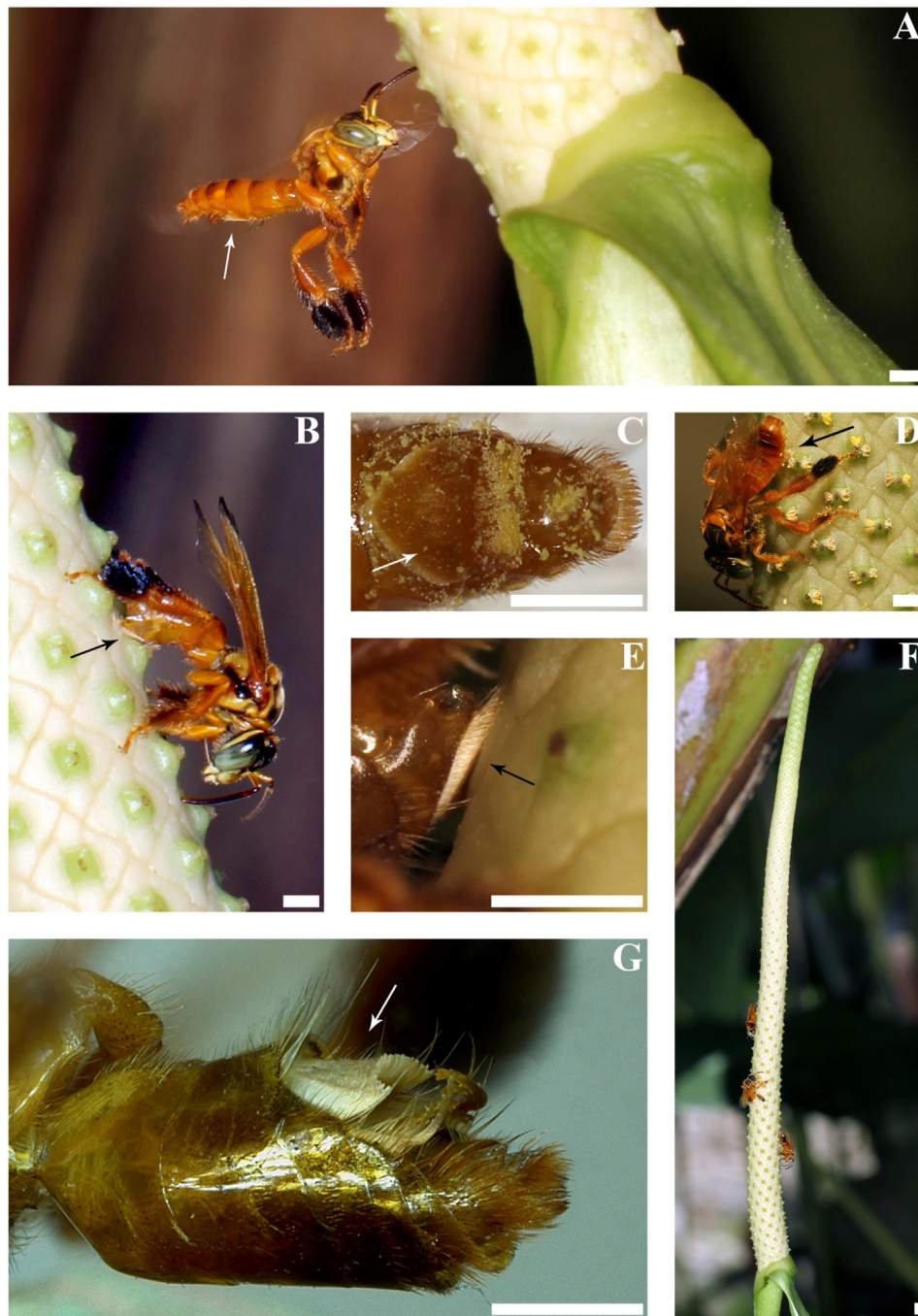


Fig. 1. Photographs of male *Anthurium acutifolium* inflorescences and male *Paratetrapedia chocoensis* oil bees. A: A male *P. chocoensis* bee approaching a scented spadix of an inflorescence of *A. acutifolium* during pistillate (female) phase. B: The abdominal mopping behavior of male *P. chocoensis* oil bees on a spadix, thereby touching a receptive stigma with the hair brush on sternum 3. C: Ventral side of the abdomen of a male *P. chocoensis* covered with pollen. D: A male *P. chocoensis* bee on a spadix of an inflorescence of *A. acutifolium* during staminate (male) phases of anthesis, touching the pollen shedding anthers. E: Pubescent region on sternum 3 pressed on the surface of *A. acutifolium* during the mopping behavior. F: A scented inflorescence of *A. acutifolium* with three male *P. chocoensis* individuals. G: Image of the abdomen of a male *P. chocoensis* in lateral view showing the conspicuous pubescent region on sternum 3. Scale bar: 1 mm.

containing compound (Table 1). The terpenoid (*E*)-megastigm-7-en-3,9-dione was the only compound present in all analysed samples, its relative amount ranging from 4% to 76%. Other major constituents in the scent bouquet of *A. acutifolium* were indole and an isomer of 9-hydroxymegastigm-7-en-3-one. Generally pistillate and staminate phase inflorescences were chemically similar and also appeared similar to the human nose.

4. Discussion

The results present strong evidence of a unique pollination system involving a typically fragrance-rewarding species of *Anthurium* and male oil bees. Male *Paratetrapedia chocoensis* possess an uncommon pubescent area on their abdomens, which during morning visits to fragrant anthetic inflorescences of *A. acutifolium* is pressed on their spadices while the bees slowly move up and down. No evidence of floral fatty oil production was found during the

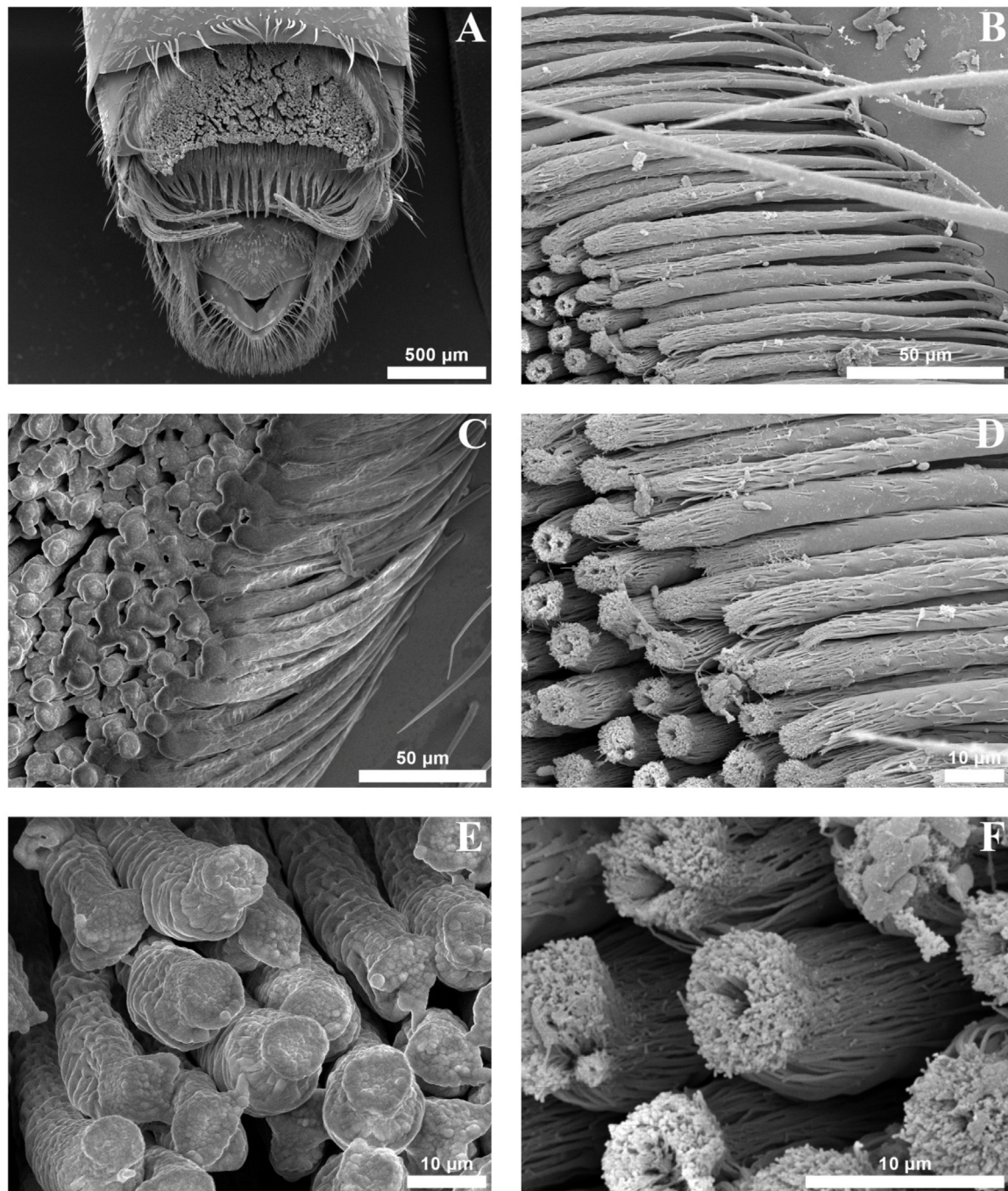


Fig. 2. Scanning electron microscopic (SEM) images of A: dorsal view of the abdomen of a male *Paratetrapedia chocoensis* bee showing the concave shaped dense pubescence on sternum 3; C, E: details of the pubescent region on sternum 3 with hair impregnated with a thin oil layer from the basis to the tip, and B, D, F: details of pubescent region on sternum 3 after solvent washing where fine hairs on the surface forming a brush towards the tip are now visible.

floral cycle of *A. acutifolium* inflorescences, nor do their flowers possess trichomal or epithelial elaiophores. The floral scent of *A. acutifolium* was constituted of indole, aliphatic esters and rare/unusual terpenoids, notably (*E*)-Megastigm-7-en-3,9-dione.

Specialized pollination by male bees is rare (Cane et al., 2011) but widespread among sexually deceptive orchids (Schiestl et al., 1999) and perfume flowers (Vogel, 1966). While sexually deceptive orchids attract males of different groups of bees by mimicking pheromones of their females, perfume flowers attract male euglossine bees through floral scents which are not only the attractive signal, but also the reward collected by these bees (Dressler, 1982; Etl et al., 1999; Roubik and Hanson, 2004; Vogel, 1966). Special-

ized pollination by male oil bees was not yet known; however, there were previous observations of male oil bees visiting the same flowers as female conspecifics, also collecting fatty oils with specialized hairs on their fore and mid basitarsi. In many of these reports, male oil bees seem to function as effective pollen vectors (Aguar and Melo, 2009; Cocucci, 1991; Friese, 1925; Vogel, 1974; Vogel and Machado, 1991; Rasmussen and Olesen, 2000; Singer and Cocucci, 1999). However, this is not the case in other reports (e.g.: Cappellari et al., 2012).

Inflorescences of *A. acutifolium* neither produce floral fatty oils nor secrete nectar, thus raising the question of which reward is sought by the male *P. chocoensis* oil bees. Pollen, which was present

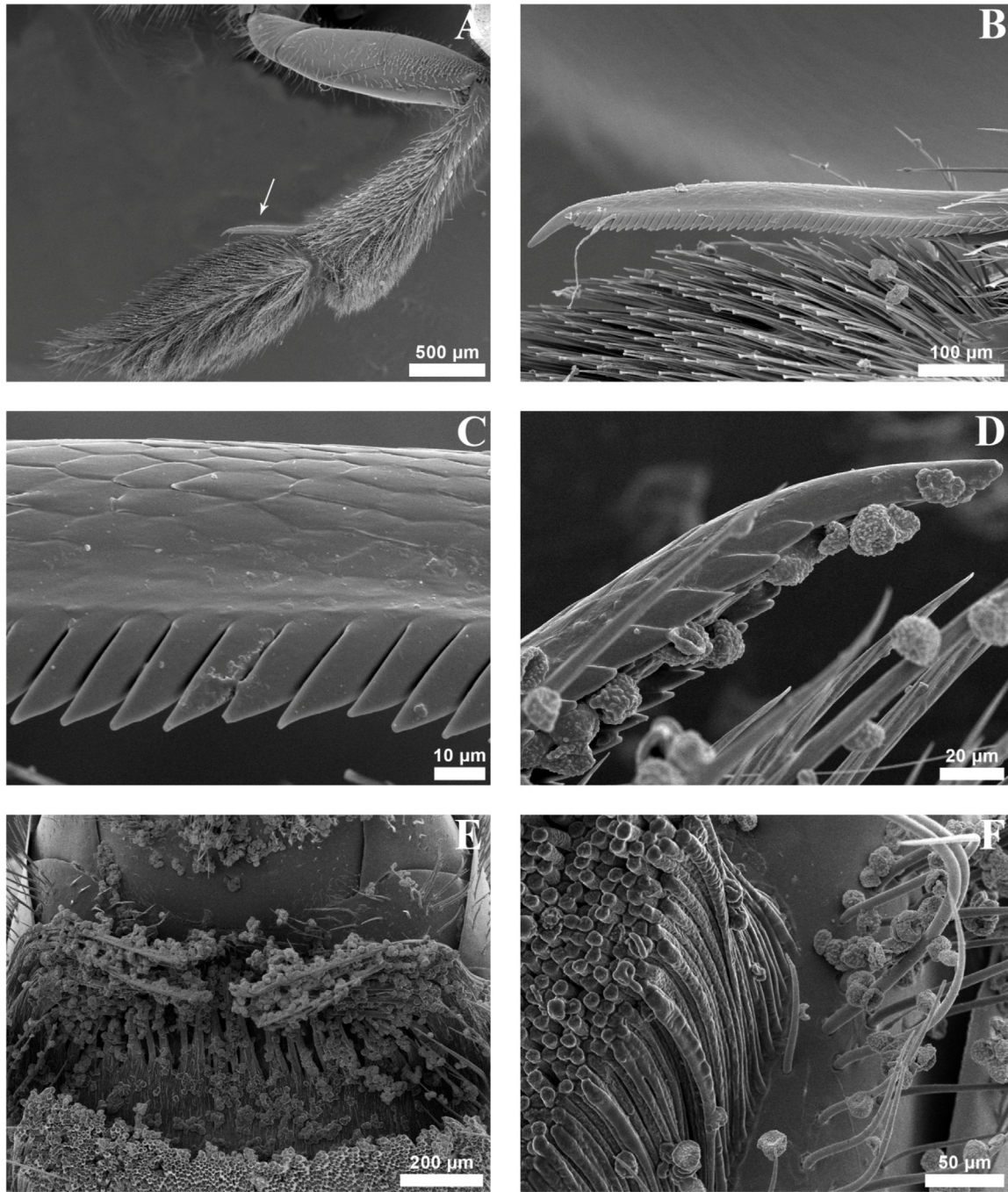


Fig. 3. SEM images of A: posterior side view of the right hindleg of a male *Paratetrapedia chocoensis*, showing the inner tibial spur (arrow); B, C: detail of the serrate shaped tibial spur showing the 10 µm wide teeth; D: pollen grains of *Anthurium acutifolium* on and in the groove of the spur; E: abdomen of a male *P. chocoensis* bee covered with pollen from *A. acutifolium* and F: detail of the outer margin of the pubescent region on sternum 3 with pollen grains trapped inbetween hairs.

during the staminate phase and has been collected by a *Trigona fulviventris* stingless bee, was also not actively gathered or eaten by male *P. chocoensis* oil bees. Instead, the spadix of pistillate and staminate phase inflorescences released its characteristic scent when visited by the bees and we hypothesize that *P. chocoensis* males visit the inflorescences to collect these scents. Thus, this pollination system seems to resemble interactions between some orchid flowers and male tephritid flies and also between perfume flowers and male euglossine bees. In contrast to male tephritid flies, which “feed” on volatiles and male euglossine bees, which collect the volatiles by their fore tarsi, male *P. chocoensis* bees mop the flowers’ surfaces using the short dense pubescence on sternum 3.

Euglossine males additionally apply lipids, originating from their cephalic labial glands, to the plant surface to dissolve and retain the volatiles (Eltz et al., 2007; Whitten et al., 1989). Male *P. chocoensis* bees seem to dissolve the perfume deposited over the spadix surface with the aid of an oil film that covers each of the specialized abdominal hairs (Fig. 2A, C, E; Fig. 3F), probably collected from other floral plant sources. Similar to other male oil bees (Cappellari et al., 2012; Vogel, 1974), *P. chocoensis* bees are likely to collect floral oils using tarsal hairs on their forelegs. The oil content is then transferred to the scopae on their hind basitarsi, where it is temporarily stored (Supplementary Fig. 6). We hypothesize that from there it is transferred to sternum 3, likely by involvement

Table 1

Occurrence, total and relative (%) amount (Mean, Min–Max) of scent compounds trapped from *Anthurium acutifolium* during pistillate (n = 2 individuals) and staminate (n = 2 individuals) phases. The Kovats retention index (RI) is also given. tr = traces (< 0.5%).

	Individual/inflorescence(s)	1/1	1/1,2	2/1	3/1	
	Sexual phase	♀	♂	♂	♀	
	Bagging time (min)	1–7	0–10	0–15	0–2	
	Sampling time (min)	2–5	2–7	2–30	2	
	Sample size	5	5	4	2	
	# of compounds	16	23	24	9	
	Total amount of scent trapped (ng/h)	474 (45–935)	1262 (672–2482)	845 (689–981)	421 (389–454)	
RI		Relative amount of scent compounds				Occurrence in n samples
	Spiroacetals					
1062	(E)-Conophthorin*	–	1 (0–2)	–	–	3
	Nitrogen-containing compounds					
1305	Indole ^a	53 (32–71)	39 (0–81)	4 (2–9)	2 (0–3)	13
	Terpenoids					
1076	p-Mentha-3,8-diene	–	–	1 (0–2)	–	1
1156	Isopulegol isomer 1	–	–	8 (0–30)	–	1
1168	Isopulegol isomer 2	–	–	5 (0–21)	–	1
1500	β-Ionone ^a	–	tr (0-tr)	–	–	1
1671	4-oxo-α-Ionone	–	tr (0-tr)	tr (0-tr)	–	4
1597	a 3-Hydroxymegastigm-7-en-9-one isomer	–	–	tr (0-tr)	–	2
1611	a 9-Hydroxymegastigm-7-en-3-one isomer	19 (6–32)	9 (tr-19)	–	40 (21–59)	12
1628	(Z)-Megastigm-7-en-3,9-dione	tr (0-tr)	tr (0-1)	tr (0-1)	–	5
1640	(E)-Megastigm-7-en-3,9-dione	9 (4–18)	36 (4–76)	37 (13–62)	27 (22–33)	16
	Aliphatics					
1607	Dodecyl acetate	tr (0-1)	–	13 (4–21)	–	5
1807	Tetradecyl acetate	tr (0-1)	–	12 (8–19)	–	5
2009	Hexadecyl acetate	–	–	2 (tr-4)	–	4
	Unknowns					
1429	m/z: 94, 79, 192, 77, 41	9 (3–15)	2 (0–7)	tr (0-tr)	24 (9–40)	12
1443	m/z: 94, 79, 77, 93, 41	1 (0–2)	tr (0-1)	–	2 (tr-3)	7
1564	m/z: 94, 43, 79, 168, 107	1 (0–4)	tr (0-1)	tr (0-tr)	2 (1–3)	10
1570	m/z: 95, 43, 109, 110, 81	tr (0-tr)	1 (tr-3)	1 (tr-2)	–	10
1666	m/z: 111, 95, 43, 149, 93	tr (0-tr)	tr (0-tr)	3 (2–4)	–	10
1716	m/z: 43, 111, 108, 94, 79	tr (0-1)	tr (0-1)	–	1 (0–3)	5
1738	m/z: 43, 82, 94, 136, 67	tr (0-tr)	tr (0-1)	–	1 (1–1)	6
1777	m/z: 82, 111, 43, 95, 67	tr (0-2)	1 (0–5)	9 (8–11)	–	7
1926	m/z: 43, 82, 96, 117, 114	8 (0–33)	9 (1–19)	3 (0–8)	1 (0–2)	12
	other unknowns ^a	tr (0-tr) ¹	1 (0-2) ⁷	2 (0-2) ⁷	–	

^a The upper script digit indicates the number of unknowns pooled.

* Identity of compounds marked with an asterisk was verified by synthetic standards.

of the tibial serrate spurs which at the same time could be used for pollen removal (Fig. 3D; Supplementary Video 4, 5). Indeed, it was known before that male oil bees carry floral fatty oils within the hairs of sternum 3, even though the bees seem not to apply these structures for collection (Antonio Aguiar unpubl. data; Vogel, 1990). If our hypothesis is true, the behavior observed in male *P. chocoensis* bees would be the reverse of the oil transferring behavior between abdomen and hind legs documented in female *Ctenoplectra* (Apidae, Ctenoplectrini). These Palaeotropical oil bees exhibit “abdominal oil mopping” (Vogel, 1981, 1990), a behavior which constitutes in pressing their abdomens on the surface of oil secreting Cucurbitaceae flowers and sweeping them from side to side to collect oils with a sternal hair brush at the end of the abdomen. Synchronously, they use comb-shaped tibial spurs to transfer the oil from their abdomens to their hind tibial scopae. A similar abdominal mopping behavior was observed in female *Tapinotaspoides* oil bees, which use hairs on their metasomal sterna to collect secretions from non-floral trichomes (Melo and Gaglianone, 2005).

The mysterious pubescent region on sternum 3 is not exclusive to *P. chocoensis*, having been observed in other congeneric male, but not female, bees. It was first reported by Stefan Vogel, who published a SEM picture of such hairs from a male individual of a *Paratetrapedia* species (Vogel, 1989). A recent phylogenetic study by Aguiar and Melo (2011, character 47: 3) showed that this character appeared only once in the evolution of *Paratetrapedia*. It occurs

in a group composed of 19 species, with secondary loss in three species. Though the use of this structure as a collecting device is described for the first time in the present study, it might not be exclusive to male *P. chocoensis*, but also used in a similar way by males of other *Paratetrapedia* species sharing this morphological trait.

Visits of male *P. chocoensis* oil bees to *A. acutifolium* inflorescences were limited to the intervals of characteristic scent emission during the pistillate and staminate phases of anthesis, strongly suggesting olfactory-mediated attraction. Whereas some of the volatile organic compounds isolated in floral scent samples of *A. acutifolium*, such as indole, are widespread across several families of flowering plants, others were only rarely described or even yet unknown as floral scent constituents (El-Sayed, 2012; Knudsen et al., 2006). Among these rare compounds were two terpenoids, (E)-Megastigm-7-en-3,9-dione and an isomer of 9-hydroxymegastigm-7-en-3-one, both abundantly present in our analysed samples. However, (E)-Megastigm-7-en-3,9-dione also occurs in small relative amounts in the floral scents of *Masdevallia laucheana*, Fraser (Orchidaceae) and in euglossine pollinated *Anthurium apaporanum*, Schultes (Kaiser, 2011; Schwerdtfeger et al., 2002), the latter additionally containing high relative amounts of numerous sesquiterpenoids, eucalyptol, eugenol, and α- and β-ionone. The absence of male euglossine bees in inflorescences of *A. acutifolium* can arguably be associated to the lack of

volatile organic compounds typically associated with their attraction (e.g., eucalyptol) in the floral scent of this species. Indeed, syntopic *Anthurium* and also *Spathiphyllum* species (their scents are not yet known) were frequently visited by male bees belonging to several species of *Eulaema*, *Euglossa* and *Eufriesea* bees (Etl et al., unpubl. data); overall diversity of euglossine bees from collections at the La Gamba Field Station is estimated at nearly 30 species (Gruber et al., 2008). Indole, a main compound in our study species, is a known euglossine attractant (Ramírez et al., 2002) and is widespread among orchid species pollinated by euglossines (Williams and Whitten, 1983). It remains to be tested whether this compound does not attract the local euglossine community or other compounds available in *A. acutifolium* exhibit repellent properties on euglossines [as demonstrated for the combination of some euglossine attractants by Williams and Dodson (1972)]. We should also emphasize that no male oil bee was ever recorded in association with any euglossine pollinated species in the study area (Etl et al., unpublished data), pointing towards an exclusive mutualistic association between *A. acutifolium* and male *P. chocoensis* oil bees.

Overall, our study offers strong support for a new pollination system mediated by male oil-bees which likely collect the uncommon volatiles, released by the plant, with an abdominal hair brush. These volatiles maybe used in male defense, territorial, courtship or mating behaviors (marking of females with antiaphrodisiacs; e.g.: Ayasse et al., 2001; see also references therein; Frankie et al., 1980). Further experiments should identify the compounds responsible for attraction of the bees, compare the scent composition of the plant with the scent composition available in the abdominal hair brush, identify the liquid (most likely fatty floral oil) in the basitarsus, and elucidate the functional significance of this newly described behavior in male *Paratetrapedia* bees.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.flora.2017.02.020>.

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