# *Coleotrype madagascarica* (Commelinaceae) visual attraction and buzz pollination by anthophorid bees in Madagascar



Miriam Rubin

UPPSALA
UNIVERSITET

# *Coleotrype madagascarica* (Commelinaceae) – visual attraction and buzz pollination by anthophorid bees in Madagascar

Miriam Rubin

Supervisors:
Prof. L. Anders Nilsson, Museum of Evolution, Uppsala University, Sweden.
Prof. Elisabeth Rabakonandrianina, Department of Ecology and Plant biology, University of Antananarivo, Madagascar.

# Table of contents

## Abstract

*Little is known about the pollination in the spiderwort family, Commelinaceae. One of the species endemic to Madagascar,* Coleotrype madagascarica *C. B. Clarke, is an extremely ostentatious plant. With visual attraction such as basally coloured apical leaves, vividly coloured petals and an astounding fuzzy spherical hair structure on the stamens. The aim of the present study was to investigate the pollination system and reveal the effective pollinator of* C. madagascarica. *Field studies in central Madagascar indicated that the flowers attract a number of species of pollen seeking bees and flies. The pollination of* C. madagascarica *relies on female bees that buzz the fuzzy bright yellow stamen filament hairs and later deposit pollen on the stigma topping the ventrally protruding hook shaped style of another flower. During a visit, the bee grabs on to the stamens and vibrate its flight muscles to eject pollen from the anthers. Anthophorid bees, especially* Amegilla *and* Pachymelus*, were the most frequent visitors and apparent pollinators.*

**Keywords**: Madagascar – Pollination – Commelinaceae – *Coleotrype madagascarica* – Buzz pollination

## Introduction

**The family Commelinaceae**
The spiderwort family Commelinaceae consists of 41 genera with approximately 650 species of mostly perennial herbs. It is widely distributed in tropical to warm temperate regions worldwide (Faden 1983, 1998). The African continent has the highest diversity, up to 40 % of all species, most of which are found in the eastern part (Faden 1983).

According to Faden (1992, 2012), Commelinaceae is sprawling or stoloniferous with erect or ascending shoots. Leaves are alternate or arranged in pseudowhorls, the sheath enclosing the stem. Inflorescences are cymose, terminal or axillary, sometimes perforating the leaf sheath when flowering (Faden 1998). Flowers are sessile with bright colours, often blue, pink, white or sometimes yellow (Perrier de la Bâthie 1938, Faden 1992). They can be either actino- or zygomorphic, commonly without floral scent (Faden 1998). The petals are hypogynous and marcescent (Perrier de la Bâthie 1938). There are six stamens arranged in two whorls, occasionally partially reduced to staminodes, with glabrous or hairy filaments. Anthers have longitudinal dehiscence, but in some taxa they are poricidal (Faden 1998).

*Pollination system*
Faden (1992, 1998) has made exhaustive descriptions of the flowers of Commelinaceae. They lack nectar, the only available reward is pollen and it is common with some anthers producing only sterile pollen as a reward. Since the flower is open for a short time, sometimes only a few hours and never more than a day, thus reducing the number of possible pollination strategies. Visual attractions are

relatively important, staminodes and vivid colours on leaves are common. Notably, *Coleotrype madagascarica* C. B. Clarke is the most extreme example, with the base of the upper leaves on flowering shoots turning a bright purple. In some genera, it is characteristic with moniliform hairs, shaped like a string of beads, attached to the stamen filaments. According to Faden (1992), these hairs can be of importance for pollination as it may attract pollinators, if vividly coloured. It could also hold pollen, or provide footing for the pollinator. The most commonly observed visitors to the flowers of Commelinaceae are bees and syrphid (hover-) flies. The only observed specialised pollination system in the family is so called "buzz-pollination". This is when a bee uses vibrations to eject the pollen from the anthers (Buchmann 1983).

*Coleotrype madagascarica*
*Coleotrype* is the only African genus of Commelinaceae with species both on the mainland and Madagascar (Faden 1983). Four species of *Coleotrype* are native to the mainland while six are endemic to Madagascar (Faden 2012). The Malagasy species share morphological traits, such as a zygomorphic corolla. Five of the stamens are more or less welded together, with the sixth being free opposite the outer petal, encircling the style (Fig. 1) (Perrier de la Bâthie 1938).

According to Perrier de la Bâthie (1938), the stems of *C. madagascarica* often reach up to 2 m and are larger than 1 cm in diameter, growing sprawling on the ground with tall ascending shoots. The leaves are lanceolate-acute, 18-20 cm long and have long closed sheaths under which the inflorescences form.  To bloom, the flowers must pierce this sheath, each inflorescence having 6-9 flowers. During flowering the base of the upper leaves turn purple. The blue petals are obtuse, 15-20 mm long, with the upper two welded together. The filaments have long, vividly yellow moniliform hair forming a "fuzz ball" around the stamens. The narrow anther loculi are more or less curved with apical poricidal dehiscence (Faden 1992). The stalkless ovary is trilocular, and the curved style is slightly thickened at the top. The seeds are ovoid and rough with small impressions.

Figure 1. Illustration of *Coleotrype madagascarica*. A: The six anthers, of which five are joined together and one is free. B: A shoot with inflorescences. C: A flower showing the stamens encircling the style. Adapted from Perrier de la Bâthie (1938).

## Buzz pollination

*Plants*

Buchmann (1983) estimated that between 13 000 – 19 000 species of Angiosperms worldwide are buzz-pollinated, also including some crops such as tomato (*Solanum lycopersicum* L.) and potato (*S. tuberosum* L.). This mode of pollination also includes some species of Commelinaceae (Buchmann & Hurley 1978, Buchmann 1983, Faden 1992, 1998). Plants that are subject to buzz pollination are characterized by vividly coloured flowers that lack nectar. Anthesis and emission of floral scent, if any, start shortly after sunrise. The plants have apical poricidal anther dehiscence with usually dry pollen (Buchmann & Hurley 1978). In some genera, like *Tripogandra* (Commelinaceae), there are two types of pollen produced. One is fertile and the other is sterile, possibly as a means of reward for the pollinator (Buchmann 1983).

*Pollinators*
As the flowers only offer pollen as reward, the majority of visitors are female bees (Hymenoptera, Apoidea), as the males only seek nectar for food (Buchmann & Hurley 1978). There is only one observed buzz visitor that is not a bee, the syrphid fly *Volucella mexicana* Macquart, a Batesian mimic of a buzzing bee that is similar in both size and colour (Buchmann 1983). The visitors are usually medium to large in size, and often oligolectic, that is collecting pollen from a particular plant family. This is probably due to the fact that visitors seem to be more selective when attracted by pollen than when attracted by nectar (Buchmann 1983). Ususally the bees are matinal, i.e. mainly foraging during the morning, which will coincide with the flowering of the plant. However, the main character for these pollinators is their behaviour during visits. As a bee lands on the flower, it will position itself over the anthers and grab on to the base of the stamens. It then contracts its large indirect flight muscles at a high frequency (50-2 000 Hz), causing vibrations to travel thru the legs to the stamens. This will quickly, in less than a few seconds, release an amount of pollen from inside the locules, causing it to project from the pores and land on the venter of the bee. This specific behaviour creates a noticeable buzz, from larger bees often audible for humans at up to 5 m distance, thus characteristic of "buzz-pollination" (Buchmann & Hurley 1978, Buchmann 1983).

Interestingly, honeybees (*Apis*) are capable of vibrating their indirect flight muscles for thermo-regulation and social communication, but they do not buzz-pollinate (Buchmann 1983). Insects that do not practise the buzzing behaviour might use other strategies to get pollen from flowers of buzz-adapted plants. Either, they can take the pollen that is left over from the legitimate pollinator, or they can chew on or "milk" the anthers to release pollen (Buchmann & Hurley 1978, Buchmann 1983).

**Aim of study**
According to Faden (1992), conducting studies of pollination biology as well as gathering basic information is needed as much is yet unknown about most members of the Commelinaceae. The aim of the present study was to reveal the basic pollination ecology and floral adaptations of *C. madagascarica* in a natural population for the first time. Since tropical primary forest is currently destroyed at an absolutely alarming rate in Madagascar and elsewhere, many more projects of this kind are urgently needed because many, or even most, of the stories of plants and their pollinators will never be told.

# Method

**Study site**
The study was conducted 25 November – 2 December and on 8 December 2014 in the Ankafobe forest in central Madagascar. Ankafobe is a 33 ha remnant of humid evergreen forest on the Madagascar central plateau (Moat & Smith 2007, Birkinshaw *et al* 2009b). It has an altitude of 1475 m, and lies approximately 130 km north of

Antananarivo (Birkinshaw *et al* 2009b). The climate in Ankafobe has been described as sub-humid without mist (Cornet 1974) (Fig. 2). The mean annual precipitation is 1850 mm, most of which falls during the hot and wet season from November to April; from May until October the weather is cool and dry (Ratsirarson & Goodman 2000).
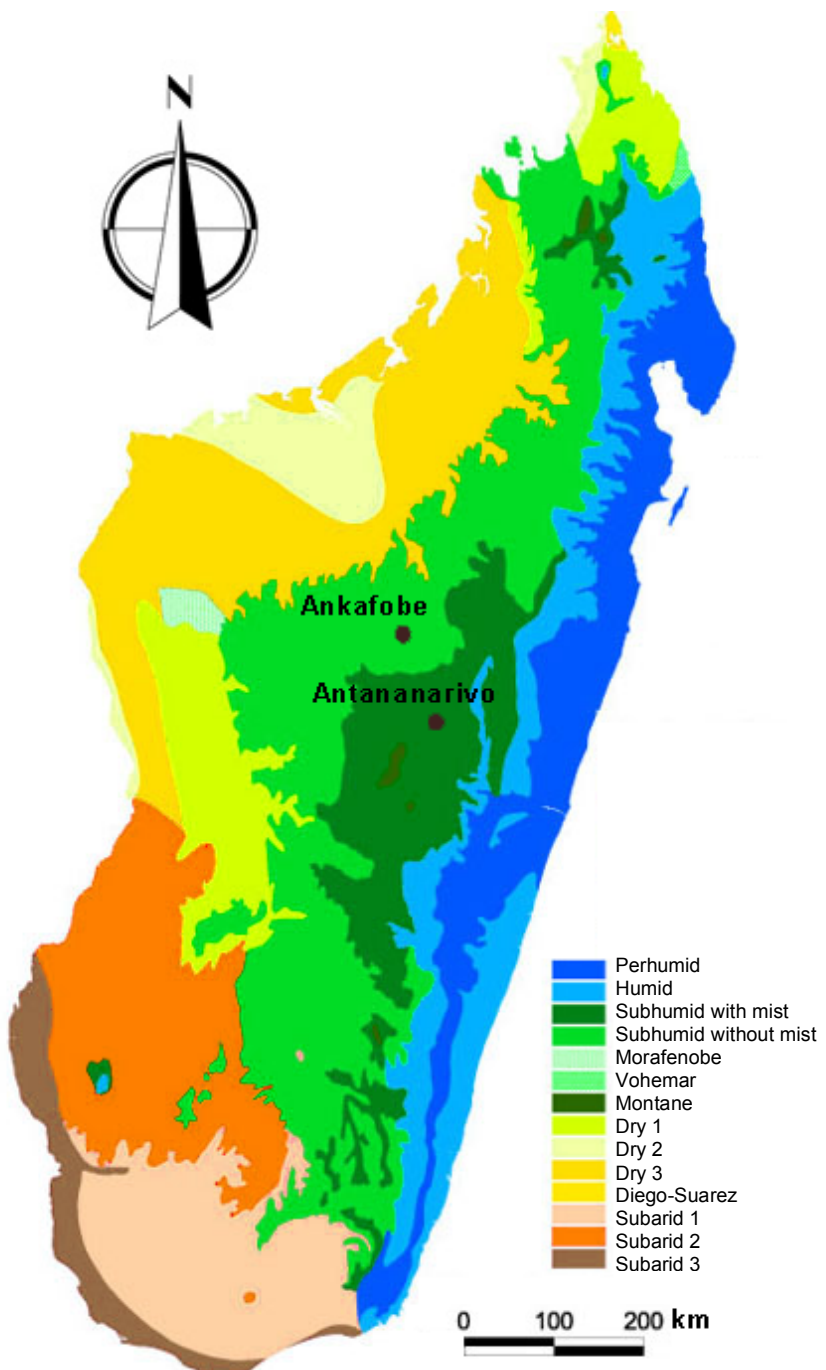


Figure 2. Madagascar's fourteen different bioclimatic zones. Ankafobe falls within the range of subhumid without mist. Adapted from Cornet (1974).

Figure 3. Ankafobe forest viewed from the top of a hill. The surrounding area consists of degraded anthropogenic grassland where the Missouri Botanical Garden is conducting *in situ* conservation by replanting locally endemic plants like *Schizolaena tampoketsana* (Birkinshaw *et al* 2009a, 2009b). The small buildings to the left are plant nurseries.
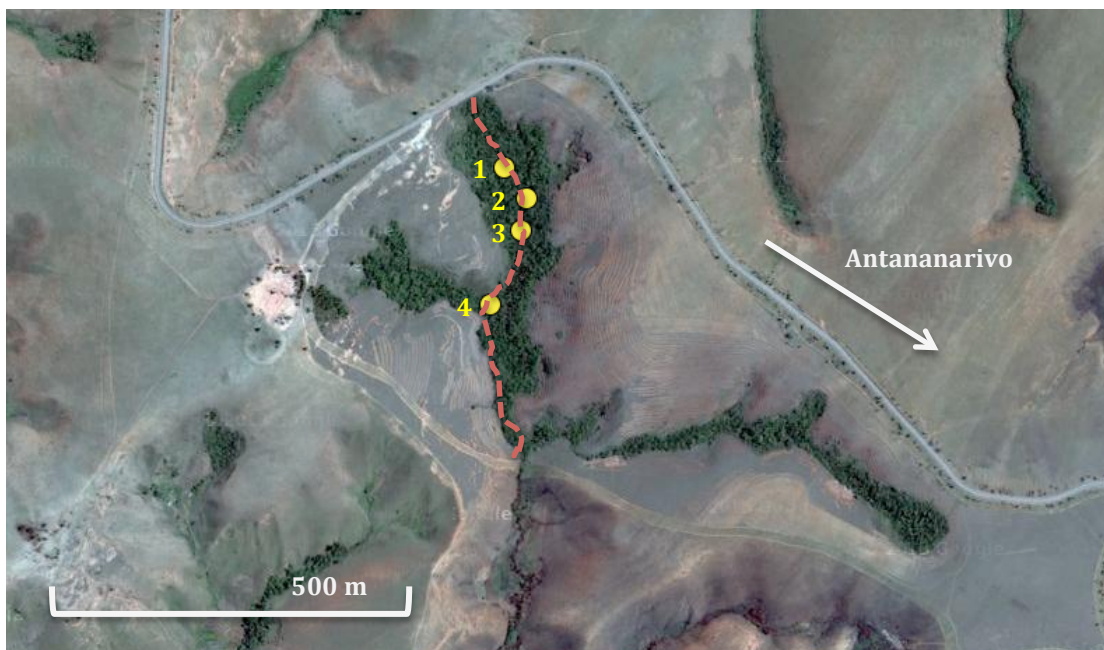


Figure 4. The severely fragmented Ankafobe forest with the four patches of *Coleotrype madagascarica* (sites 1-4 from the top) marked in yellow. The dashed red line is an approximation of the pathway leading through the forest. The surrounding land is degraded anthropogenic grassland. Scale: 500 m. The nearby road leads southeast to the capitol. Satellite image from Google maps.

The vegetation surrounding Ankafobe consists of mostly anthropogenic plateau grassland dominated by species such as *Loudetia simplex* (Nees) C. E. Hubb., *Trachypogon spicatus* (L.) Kuntze and *Aristida* spp (Poaceae) (Fig. 3). It also has

some scattered elements of woods but the open landscape is maintained by fires and grazing (Moat & Smith 2007, Birkinshaw *et al.* 2009b). Wildfires occur annually, and the forest was subject to wildfire as recently as in October 2014, only a few weeks before the study (Birkinshaw & Raharimampionona 2014).

Inside the Ankafobe forest there is a steep forest slope down into a somewhat marshy valley with some clearings. There is also a small winding path leading from the main road (RN 4) down to and then along the moist bottom with forest. There, plant diversity is large for such a small area, with taxa such as *Pandanus pulcher* Martelli (Pandanaceae), *Ophiocolea* (Bignoniaceae), *Ravenea madagascariensis* Becc. (Arecaceae), *Chrysophyllum boivinianum* (Pierre) Baehni (Sapotaceae), *Desmodium* (Fabaceae) and *Symphonia* (Clusiaceae). Indeed, some of the species found there are endemic to the area: *Dypsis decipiens* (Becc.) Beentje & J. Dransf. (Arecaceae) (Rakotoarinivo & Dransfield 2012)*, Dombeya ankazobeensis* Arènes (Malvaceae) and the critically endangered *Schizolaena tampoketsana* Lowry, G. E. Schatz, J.-F. Leroy, & A.-E. Wolf (Sarcolaenaceae, *Sohisika* in Malagasy) (Birkinshaw *et al* 2009a and b).

Patches of *C. madagascarica* were found at four separate sites (S18° 06' 09.6", E47° 11' 13.5"; S18° 06' 11.2", E47° 11' 14.7"; S18° 06' 12.9", E47° 11' 14.4" and S18° 06' 16.8", E47° 11' 12.7") but it also grew scattered along the path. Each of the patches was used in the project, referred to as site 1-4, respectively (Fig. 4). There are no earlier reported records of *C. madagascarica* from this location (E. Rabakonandrianina pers. comm. 2014).



Figure 5. Patch 2 of *Coleotrype madagascarica*, as seen from the path. The patch grows in the swampy understory of the forest. Reproductive shoots are easily spotted with their bright purple apical leaves.

**Reproductive population and phenology**

The sprawling way *C. madagascarica* grows made it difficult to distinguish autonomous individuals (Fig. 5). At each of the sites 1-4, a rectangular plot containing representative shoots (i.e. probably mostly ramets) was created. It measured 2 x 5 m, 3 x 5 m, 2 x 3 m and 4 x 5 m respectively, due to the patches being of different sizes. Five 0.5 x 0.5 m squares were then chosen at random in each plot. Within each square, all shoots were counted and classified as either vegetative (= leaves exclusively green) or reproductive (= apical leaves with more or less purple base). Throughout the study, all shoots that had purple apical leaves were regarded as a reproductive shoot. This was done on 8 December, in order to estimate the size of the population in terms of shoots.

To examine the reproductive phenology of the population, the plots at sites 1 and 2 were used. By sampling random points in each plot and choosing the closest reproductive shoot yielded 25 shoots at each site. For each, the numbers of inflorescences and purple-coloured leaves were counted. The inflorescences were counted from the bottom-up and, for each, the numbers of visible buds, flowers in bloom, wilted flowers and visible fruits were noted. This was repeated three times during the study, 26 November and the 1 and 8 December.

**Flower morphology**

Shoots with flowers in bloom were sampled to determine the size of the flowers and their reproductive parts. All morphological measures (Fig. 6A & B) were taken using a digital calliper with 0.1 mm precision. To examine the number and extent of purple apical leaves they were counted from the top down and total length and length of purple colour along the central vein (Fig. 6C) were measured with a measuring tape. Anthesis was monitored to determine at what time during the day the flowers became available to pollination. This was done by marking large buds, giving them an identification number, early on the day of flowering and measuring the flower diameter (Fig. 6D) of the opening flower with a digital calliper at half-hour intervals between 07:30 and 11:00 a.m.
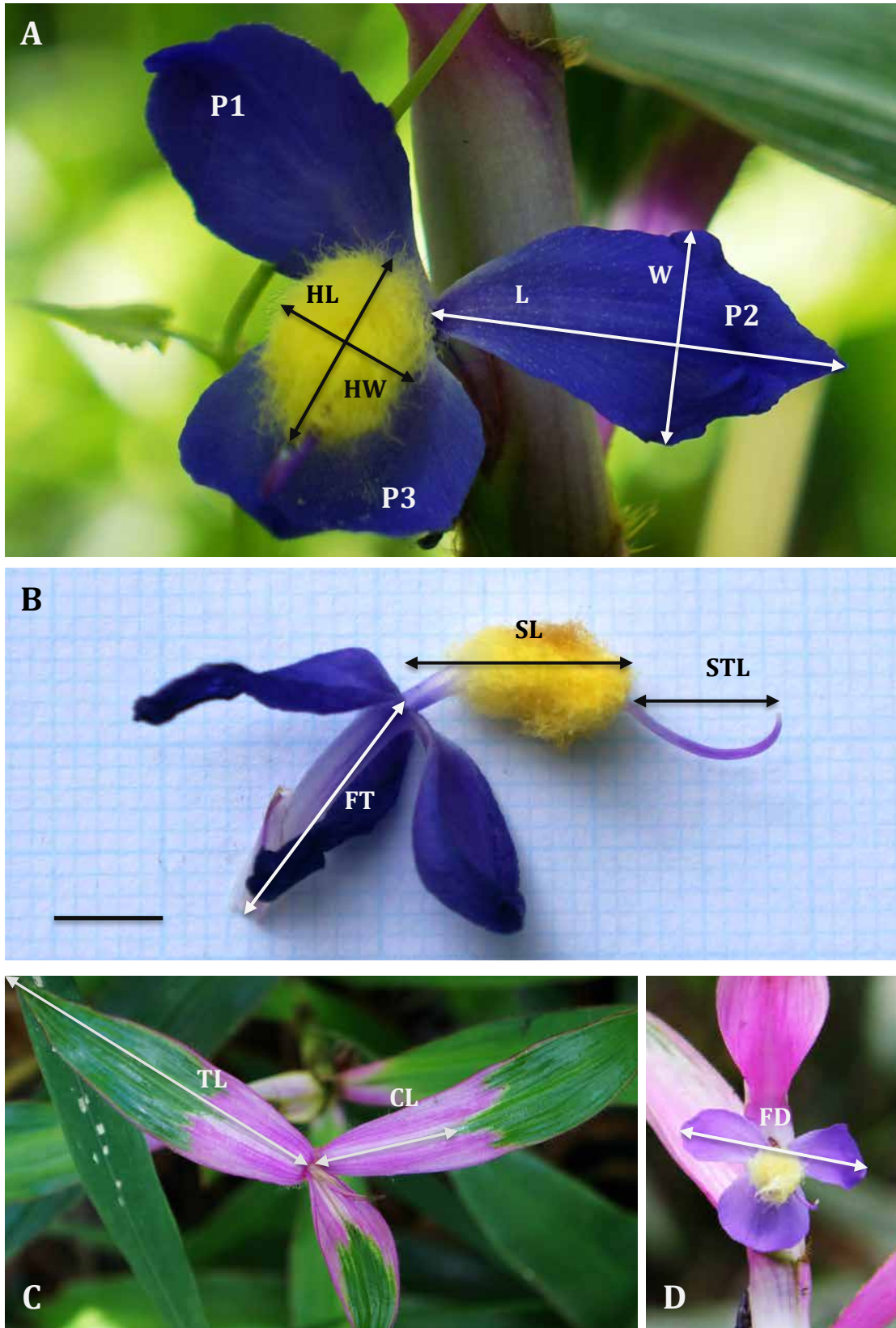
Figure 6. Measurements of the morphology of *Coleotrype madagascarica*. A: Flower, frontal view. Width (W) and length (L) of petals one (P1), two (P2) and three (P3). Width (HW) and length (HL) of the yellow hair structure. B: Flower, lateral view. Length of longest stamens (SL), style (STL) and flower tube (FT). Scale: 5 mm. C: Purple apical leaves, dorsal view. The total length of leaf (TL), and length of purple colour (CL) measured along the mid vein. D: Flower, frontal view. Flower diameter (FD).

**Visitor observations**

Four people made observations between 25-30 November 2014, between 07:30 a.m. and 15:00 p.m. for a total of 95.5 man-hours. Since weather may affect insect activity and therefore visitor frequency, simple weather data was documented. The same person recorded temperature and cloud cover, once an hour throughout the study to enable an assessment of visitation data. During a flower visit, the observer noted time and appearance of the visitor in addition to characteristics such as colour and size. Detailed notes were taken on the number of shoots and flowers visited and the behaviour of the visitor, including how long the visitor stayed on the flower or if it touched the floral reproductive parts. If the visitors did repeated visits to the same flower, each was counted as a separate flower visit. The most frequently visiting insect species were subject to sampling for identification and documentation. In addition to pressed samples of shoots of *C. madagascarica*, the insects have been deposited as voucher specimens in the Museum of Evolution (UUZM), Uppsala University.

**Breeding system**

Pollination experiments were conducted to evaluate how dependent *C. madagascarica* is on a pollinating agent to produce fruit. Thirty shoots, based on their prevalence of visible buds, were chosen among the four sites. Three buds on each shoot were enclosed with a fine-mesh net (1x1 mm) to exclude visitors throughout anthesis. On the day of anthesis the flowers were subject to one of three treatments: (1) constant isolation, (2) artificial self-pollination, and (3) artificial cross-pollination. In the cross-pollination treatment, the pollen of the donor was taken from a different patch/site. If highly dependent on a pollinator (e.g. the plant is self-incompatible), flowers that are subject to artificial self-pollination or in constant isolation should not produce fruit. The result was recorded on 8 December. A subset of shoots with no treatments was also collected to get an estimate of the natural fruit set.


## Results

**Population size and phenology**

Between 81-86 % of the shoots in patch 1-3 were vegetative (Table 1). In contrast, patch 4 had an overwhelming majority of reproductive shoots (91 %). However, patch 4 had an overall low density of 1.1 shoots / $m^2$. The highest density was in patch 1, with 3.2 shoots / $m^2$.

Table 1. The total number of vegetative and reproductive shoots of *C. madagascarica* at each of the four study sites. Area refers to the size of the plot.

| Site | Vegetative shoots/$m^2$ | Reproductive shoots/$m^2$ |
|:----:|:-----------------------:|:-------------------------:|
| 1    | 2.6                     | 0.6                       |
| 2    | 1.9                     | 0.3                       |
| 3    | 3                       | 0.5                       |
| 4    | 0.1                     | 1                         |

*Phenology*

Overall, the number of wilted flowers was substantially larger than the number of flowers, buds and fruits (Fig. 7). At the first census, there was an average of 12.24 ± 8.92 (mean ± SD) wilted flowers per shoot. The number had increased on 2 December (17.78 ± 12.71) but by 8 December it had decreased again (12.44 ± 11.01). The number of open flowers per shoot stayed rather constant: 0.54 ± 0.78, 0.50 ± 0.61 and 0.58 ± 0.88, respectively. On one shoot there was most often one, sometimes two and never more than three flowers in bloom at the same time.
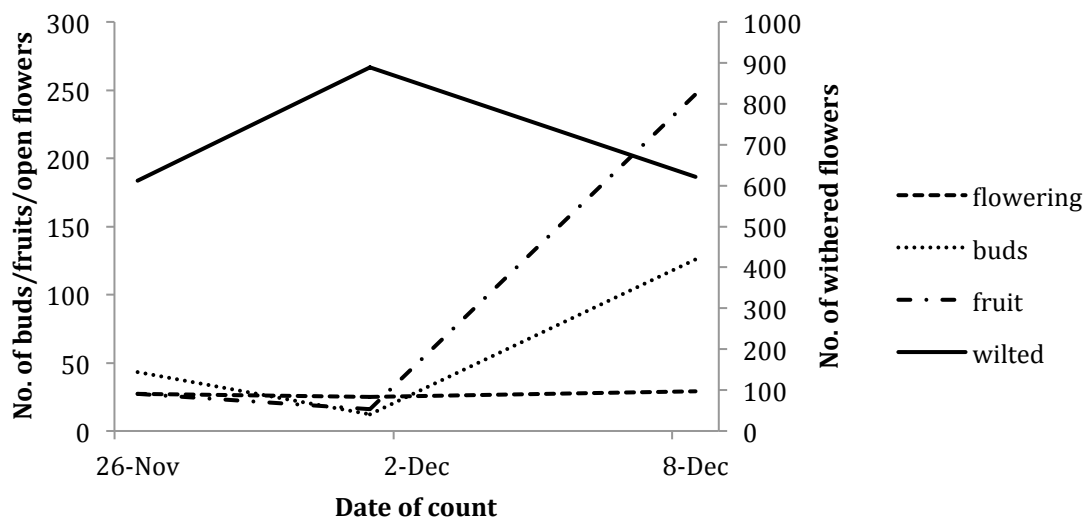


Figure 7. The total number of visible buds, open flowers, visible fruit and wilted flowers on three dates during the flowering 2014 ($N = 50$).

The number of fruits on the other hand, was low at first (0.86 ± 1.45). After an initial small decrease (0.24 ± 0.71), it increased (2.52 ± 2.95). The same pattern was seen in the number of buds (0.54 ± 0.78; 0.32 ± 0.76; 4.94 ± 5.56). The number of wilted flowers did not increase over time. The number of buds increased, probably due to the fact that they develop inside the leaf sheath and thus were not possible to count until they emerged.

**Morphology**

Flowering seemed random, with no particular order to the position of which flowers were in bloom. The corolla is zygomorphic with no visible calyx (Fig. 6). Petals 1 and 2 are oblong, and even though they differed slightly among shoots, they had almost identical means. The third petal was slightly shorter but wider, forming a bottom lip. The yellow hairs on the stamen filaments formed an oblong spherical structure around the upper part of the stamens, including the anthers. The hook-like style protruded almost a centimetre in front of the stamens (Table 2). Before flowering, the bud protrudes from the leaf sheath so that during bloom, the corolla is visible on the outside while the flower tube remains hidden inside the sheath.

12

On average, shoots had 7.5 ± 1.74 (mean ± SD, $N = 22$) basally coloured leaves at the top. The leaves decreased in size towards the top while the proportion of purple colour increased (Fig. 8). The largest number of purple coloured leaves was 11, but this was only found on two shoots. None had less than five coloured leaves.

Table 2. Biometry of flower parts (mm). $N = 23$, except for Flower tube length where $N = 14$. Measured according to Fig. 6.

| Character | Mean | SD | CV (%) | Range |
|---|---|---|---|---|
| Petal 1 length (L) | 15.6 | 1.7 | 10.9 | 12.8 – 18.7 |
| Petal 1 width (W) | 6.7 | 1.7 | 24.9 | 4.0 – 9.7 |
| Petal 2 length (L) | 15.5 | 1.3 | 8.6 | 12.5 – 17.8 |
| Petal 2 width (W) | 6.8 | 1.8 | 25.7 | 4.1 – 11.1 |
| Petal 3 length (L) | 14.3 | 1.7 | 11.6 | 10.9 – 16.3 |
| Petal 3 width (W) | 9.9 | 1.2 | 11.8 | 7.4 – 12.3 |
| Stamen hair ball length (HL) | 9.0 | 0.9 | 9.7 | 7.3 – 10.9 |
| Stamen hair ball width (HW) | 5.6 | 1.5 | 25.8 | 3.7 – 9.5 |
| Longest stamen length (SL) | 7.5 | 0.7 | 18.5 | 5.9 – 8.6 |
| Style length (STL) | 10.8 | 2.0 | 9.4 | 7.3 – 16.2 |
| Flower tube length (FL) | 17.2 | 2.0 | 6.9 | 23.7 – 29.8 |
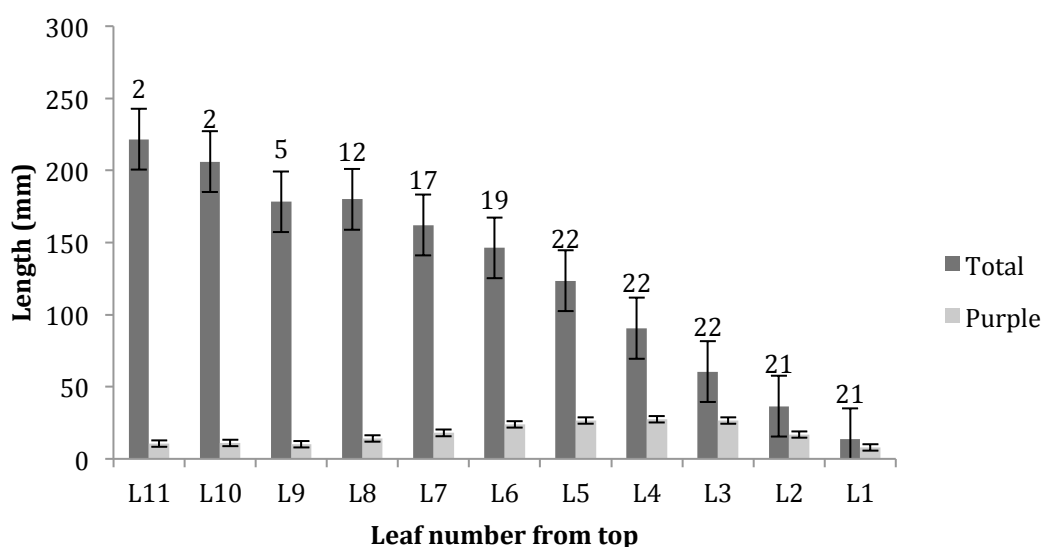


Figure 8. The mean of total leaf length and the length of purple on each leaf, with standard error bars. The sample size ($N$) for each leaf is stated above the column, decreasing toward the lower leaves. One shoot had a damaged top, hence the lower sample size for L1-2.

*Anthesis*

The flowers of *C. madagascarica* opened during the morning (Fig. 9). All the flowers were closed buds at 07:30 in the morning. At 10:00 all of them were fully opened. The flowers were ephemeral and wilted during late afternoon.
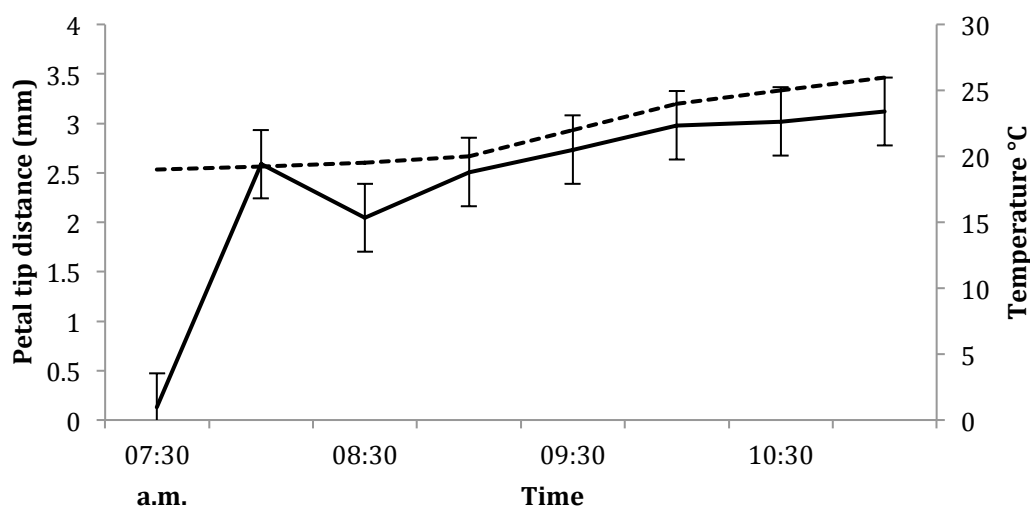


Figure 9. The flower diameter measured as the distance between the tips of petals 1 and 2 in mm (solid line) and temperature (dashed line), measured over time ($N = 15$).

**Flower visitors**

The two most common visitor species observed were the anthophorid bees *Amegilla antimena* and *Pachymelus limbatus* (Table 3), with a relative visiting frequency of 40 % and 33 %, respectively. These two species vastly outnumbered the other visitors, of which a majority were bees: *Sphegocephala philanthoides* (16 %), *P. micrelephas* (3.4 %) and *Xylocopa calens* (2.5 %). All these bees are common and polylectic (Pauly *et al*. 2001, L. A. Nilsson pers. comm.), meaning they collect pollen from several species of plants in two or more families. Apart from the bees, there were three species of Diptera: two species of *Eumerus* (2.5 %) and *Stomorhina lunata* (0.6 %). As the *Eumerus* flies were not identified to species (possibly new), their visits have been pooled. Malagasy *Eumerus* species and especially *Stomorhina* are known to visit other flowers and frequently eat pollen (L. A. Nilsson pers. comm.). All visitors were still active on days when the vegetation was wet due to rain the previous night. During heavy overcast, however, their activity decreased.

One or two species of wasp also visited *C. madagascarica* a few times (1.8 %). They were never caught, and so could not be identified further. A few species of large butterflies sometimes flew across the sites. They frequently passed by site 2 that was more open and sun-exposed than the other three. Seemingly attracted by the purple apical leaves, they fluttered across the patch but never visited the flowers.

14

Table 3. The observed flower visitors on *C. madagascarica* at Ankafobe. The buzz visits presented are cases in which they were undisputedly audible.

| Visitor | No. patch visits (freq. %) | No. buzz visits (freq. %) | No. shoots visited (freq. %) | No. flowers visited (freq. %) |
|---|---|---|---|---|
| **Hymenoptera** | | | | |
| Apidae | | | | |
| *Pachymelus limbatus* Ss. ♀ [P] | 108 (33) | 11 (69) | 1081 (46) | 1300 (44.7) |
| *Amegilla antimena* (Ss.) ♀ [P] | 131 (40) | 1 (6) | 949 (41) | 1264 (43.4) |
| *Sphegocephala philanthoides* Ss. ♀ [P] | 52 (16) | - | 165 (7) | 188 (6.5) |
| *Pachymelus micrelephas* Sm. ♀ [P] | 11 (3.4) | 4 (25) | 66 (3) | 78 (2.7) |
| *Xylocopa calens*\* Lep. ♀ | 8 (2.5) | - | 46 (2) | 56 (1.9) |
| Hymenoptera unid. spp.\* | 6 (1.8) | - | 10 (0.41) | 13 (0.45) |
| **Diptera** | | | | |
| Syrphidae | | | | |
| *Eumerus* unid. spp. ♀ | 8 (2.5) | - | 9 (0.4) | 9 (0.3) |
| Calliphoridae | | | | |
| *Stomorhina lunata* (F.) ♀ | 2 (0.6) | - | 2 (0.09) | 2 (0.07) |

\* Only identified in the field, not captured.

[P] Visitor was observed with pollen load on hind tibiae.

*Visitor behaviour*
*Pachymelus limbatus* is a slightly larger than medium sized bee, ca 17 mm in length (Pauly *et al*. 2001)(Fig. 10). When approaching a site it seemed attracted by the purple leaves. It hovered over them before visiting the flowers, sometimes flying close to shoots without any flowers in bloom. Flying across the entire patch, it visited all open flowers. The visits on each flower were short, generally only 1-2 seconds. On a number of occasions it was heard and seen "buzzing" and it often carried pollen on its hind tibia. The peak of activity occurred early, at approximately 09:30, with an instant decrease around 09:45. After this dip, it stayed active until mid afternoon (Fig. 11). *P. limbatus* was frequently seen immediately revisiting a flower it had just visited. This behaviour could be repeated up to three or four times.

In general, *Amegilla antimena* behaved much like *P. limbatus*, but is considerably smaller, 10-13 mm (Pauly *et al*. 2001)(Fig. 10). It seemed attracted by the purple leaves, approaching them and hovering above them before visiting the flowers, only spending approximately 1-2 seconds per flower. It was seen "buzzing" only once. *A. antimena* was so small that it in fact crawled on the hair structure, and sometimes seemed too small to be able to hold on properly. Activity was high throughout the morning, with one peak at 10:30 and one at 13:00 with a dip in between (Fig. 11).

Figure 10. The three flower visitors of *Coleotrype madagascarica* that were observed buzzing, from top left: *Pachymelus limbatus*, *Amegilla antimena* and *P. micrelephas*. Scale: approximately 1 cm.

Of all the visitors, *Sphegocephala philanthoides* was both the smallest and the fastest, often flying rapidly over the patch, only visiting a few flowers. The time spent on each flower varied much, 2-10 seconds. It was seen crawling on the hairs but not buzzing.

With ca 30 mm in length, *Pachymelus micrelephas* is substantially larger than its relative *P. limbatus* (Fig. 10), it is in fact one of the largest anthophorids in the world (Pauly *et al.* 2001). On *C. madagascarica* it was confirmed to buzz on 4 visits of the total 11, comprising 25 % of the total buzz visits. However, the visits were fast, only about 1 second per flower. Like *P. limbatus*, it often did repeated visits to the flowers and had several peaks in its activity (Fig. 11).
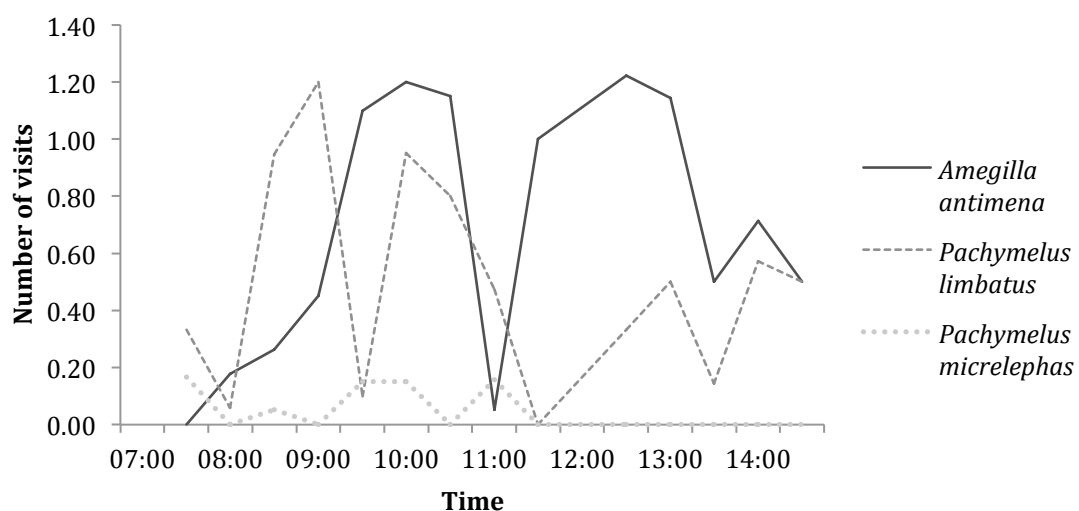


Figure 11. The total number of visits during the day for the three visitors that were observed buzzing: *Amegilla antimena*, *Pachymelus limbatus* and *P. micrelephas*. The observations have been scaled against the number of times each half-hour interval was observed.

16

Of the more infrequent visitors, there was *Xylocopa calens*. It is a large bee, 19 mm in length (Pauly *et al.* 2001). It was only observed visiting *C. madagascarica* on 8 occasions. During a visit, it covered the entire site, visiting all open flowers for approximately 1 second each. Some species had a tendency to stay a longer time on each flower during a visit, the most extreme being *Eumerus* spp. that only visited one flower during approximately 5 minutes probing the yellow hairs for loose accessible pollen. It also had a distinct behaviour; flying back and forth in front of the flower's hair structure. Most likely, it was feeding on left over pollen stuck in the yellow stamen hairs. *Stomorhina lunata* seemed to forage in a similar manner as *Eumerus*.

**Pollination experiments**
Of the 17 inflorescences with a flower subjected to cross-pollination only four produced fruits, giving a fruit set of 23.5 % (Table 4). The self-pollinated and bagged treatments had 3 and 2 fruits, respectively. However, due to the fact that the fruit matures inside the leaf sheath it was not possible to say which flower of each inflorescence that indeed had produced a fruit. The natural fruit set was 31.3 %.

Table 4. The resulting fruit set of the experimental pollination treatments. The natural fruit set is also reported. * Percentages are somewhat uncertain (see text).

| Treatment | No. flowers | Fruit set (%) |
|---|---|---|
| Cross-pollination | 17 | 23.5* |
| Self-pollination | 16 | 18.8* |
| Bagging | 19 | 10.5* |
| Natural fruit set | 83 | 31.3 |

## Discussion

**Breeding system**
Because of the way the fruit develops inside the leaf sheath on *C. madagascarica*, the results of the breeding experiments are inconclusive. After the wilted flowers have fallen off, it is very difficult to distinguish which of the flowers in the inflorescence that was subject to treatment and therefore which flower that produced fruit. According to the literature (Perrier de la Bâthie 1938, Faden 1998), all stamens are fully developed and their pollen supposedly fertile, but actual variation in amount of pollen and fertility among the six stamens have not been tested; it is possible that, for instance, some anthers produce less fertile pollen. Partially sterile stamens do occur in the family as a means of reward for the pollinator, and to avoid wasting fertile pollen (Faden 1992, 1998).

Future tests should bag entire shoots before any inflorescence has started to flower and only perform treatments on one flower per inflorescence so as to avoid confusion when recording the results. It would also be of importance to find a way to properly mark experimental flowers individually for a longer period without destroying them,

as they are quite soft and fragile and tend to fall off some time, approximately a week, after wilting.

**Visual attraction in the Commelinaceae**
The attraction of pollinators to flowers in general is a complex compound of characters (Scogin 1983). In the Commelinaceae family, the attraction is primarily visual as most species lack floral scent (Faden 1992). In general, bees are attracted to vivid colours, such as bright blue or yellow but flowers sometimes have patterns in ultraviolet wavelengths (UV), visible to bees but not perceivable by humans (Scogin 1983). There is, however, little investigation about UV-patterns in Commelinaceae (Faden 1992). One of the few studies was conducted by Simpson *et* al. (1986): they investigated the UV-patterns in *Tinantia anomala* C. B. Clarke and found that the upper anthers (staminodes) and their surrounding hairs absorb UV-light, thereby contrasting the petals behind them, which reflect UV-light. In general, the contrast between the different floral parts and between the flower and the background are important as they make the flower stand out from its surroundings and more attractive from afar (Scogin 1983). The yellow hairs on the stamen filaments of *C. madagascarica* create a sharp contrast against the blue petals. Faden (1992) pointed out *C. madagascarica* as an extreme example of visual attraction at a distance due to its basally coloured apical leaves. Buzz pollinated flowers like *C. madagascarica* have relatively few anthers that are arranged around the style and are often an eye-catching yellow that contrasts the corolla (Faden 1983). One would expect it to be difficult for visitors to collect pollen from *C. madagascarica* due to the positioning of the flowers but, according to Buchmann & Hurley (1978), floral position does affect the pollen release.

The hairs on the stamen filaments may have several purposes (Faden 1992). First, since the only available reward offered is pollen and there is no floral scent, the androecium is the primary attractant (Faden 1992). Due to the poricidal anthers, the pollen itself will not be visible to pollinators (Lunau 2006). The hairs may aid in this attraction as they give an impression of a large amount of pollen (Faden 1992). This could be considered as a form of deceit as many different kinds of bees can be attracted to and explore the flowers, but will not be able to access the pollen if they lack the ability to buzz (e.g. honeybees). In those cases, the yellow stamen hairs will act as a pollen "dummy", attracting the insects by giving them the impression of a large amount of accessible pollen even though they in fact cannot access it without the proper tools: buzzing. Second, another purpose of these hairs could be to prevent pollen thieves and visitors, even legitimate pollinators, from collecting the pollen too efficiently (Faden 1992). If the pollen is more difficult to access, it will require more visits, which in turn will increase out-crossing (Faden 1992). As the hairs will catch some shed pollen they could also play a part in transferring it to the venter of the pollinator (Faden 1992). In the genus *Cyanotis* they are essential in this aspect as the anthers have basal poricidal dehiscence and the pollen would be lost if not for the

hairs (Faden 1992). Third, the hairs can provide footing for smaller visitors, like small bees and syrphids, that need to land on the flower to be able to feed (Faden 1992).

**Legitimate pollinator**

With 44.7 % of all flower visits, *Pachymelus limbatus* was the most frequent visitor, responsible for 69 % of all buzz visits. *P. limbatus* is a bee that is common in the forests of the central plateau and eastern Madagascar (Pauly *et al*. 2001). Its females are known to collect pollen from *Dichaetanthera cordifolia* Baker (Melastomataceae) and perhaps occasionally *Aristea kitchingii* Baker (Iridaceae) (Pauly *et al*. 2001). Its size, ca 17 mm (Pauly *et al*. 2001), indicates that its venter, with pollen from previous visits, should touch the style of *C. madagascarica*, thereby pollinating it. The fact that the style is shaped like a hook might aid in transferring pollen from the bee to the stigma. The frequent repeated visits indicate that *P. limbatus* perceived that it was able to acquire pollen and tried to collect more. Reasonably, therefore *P. limbatus* is a likely primary pollinator.

The second most frequently observed visitor was *Amegilla antimena*, 43.4 % of all flower visits. Common in central Madagascar, it is a confirmed buzz pollinator of the genus *Dichaetanthera* (Melastomataceae) (Pauly *et al*. 2001) and has been seen performing buzz visits to other plants (L. A. Nilsson pers. comm.). *A. antimena* seems too small to be able to pollinate *C. madagascarica* as it did not touch the style during visits. When visiting a flower, *A. antimena* was able to crawl on the yellow stamen hairs, and it was only observed performing buzz pollination once. Both *P. limbatus* and *A. antimena* had a dip in their visitation frequency, which may reflect that a majority of the female bees tend to return to the nest with a load of collected pollen after a certain foraging time, thus like waves.

*P. micrelephas* is endemic to Madagascar with a distribution along the forests of the central plateau. It is a pollinator of several plant species of different families, for instance: *Solanum erythracanthum* Bojer (Solanaceae), *Amphorocalyx multiflorus* Baker (Melastomataceae) and *Campylospermum deltoideum* Tieghem (Ohnaceae) (Pauly *et al*. 2001). As a pollinator, it often uses buzzing (Pauly *et al*. 2001). Even though *P. micrelephas* only had a patch visit frequency of 3.4 %, it was responsible for 25 % of the recorded buzz visits. Its large size and rather few visits suggest it might be a secondary pollinator.

*Sphegocephala philanthoides* is a confirmed buzzing species (Pauly *et al*. 2001) but was never seen buzzing on *C. madagascarica*. The behaviour of *S. philanthoides* suggests that this bee is suboptimal as a pollinator. When visiting a patch, it only visited few flowers, approximately 3-4. The varying time they spent on the flowers, 2-10 seconds, further suggests this limited role. For all visitors, the observation distance may have affected the number of detected buzz visits. Especially for smaller visitors, like *A. antimena* and *S. philanthoides*, the buzz would likely not be audible at distances more than 1-2 m.

*Xylocopa calens* is very common throughout Madagascar and also found on the mainland of Africa; it is even found in severely degraded habitats. It is a very common pollinator on a number of plants from different families and is very often seen in gardens (Pauly *et al*. 2001). Only visiting *C. madagascarica* on 8 occasions, and never seen buzzing, it can be assumed that *X. calens* is a suboptimal pollinator.

The other visitors, *Eumerus* spp. and *Stomorhina lunata* are likely pollen thieves. They had very few visits to the patches and only visited 1-2 flowers per visit, spending up to five minutes on it. It is common for Malagasy syrphids to eat pollen, and *Stomorhina lunata* is a very common species of bluebottle in Madagascar that is also often seen eating pollen of many different plant species (L. A. Nilsson pers. comm.).

**Conservation**

Madagascar is considered one of the worlds top biodiversity hotspots based on its rate of endemic species (Myers *et al.* 2000). The island is home to a spectacular eleven families and 310 genera of endemic plants, 3.2 % of the world's known plant species (Myers *et al.* 2000). Myers *et al.* (2000) rated Madagascar as one of the "hottest" hotspots, not only based on its high rate of endemism, but due to its extensive loss of primary vegetation. Today only 9.9 % of Madagascar's primary forests remains (Myers *et al.* 2000). A majority of the remainder consists of species poor secondary grassland (Du Puy & Moat 1998). The grassland is prone to erosion and burnt annually to provide grazing for cattle (Du Puy & Moat 1998). This is the kind of grassland that surrounds the Ankafobe forest where Missouri Botanical Garden is conducting a project of reforestation (Birkinshaw *et al.* 2009b). Together with a local non-governmental organisation and the university of Antananarivo they are working towards replanting the native flora (Birkinshaw *et al.* 2009b). One of the most important species in this project is the critically endangered *Schizolaena tampoketsana* as Ankafobe encompasses a majority of the population (Birkinshaw *et al.* 2009b). There is very little information about the Commelinaceae in general (Faden 1992), and no available up to date information about either the distribution or conservation status of *C. madagascarica*. It is therefore difficult to assess the future prospects for this species. As this study was aimed to increase the knowledge about *C. madagascarica*, it might be of use for future assessments of conservation.

**Conclusion**

The time of anthesis, visual attraction signals and floral morphology imply that *C. madagascarica* is pollinated by medium to large sized sized bees. Due to its poricidal anthers, the pollination system relies on buzz visits by bees. When visiting a flower, the pollinator grabs on to the anthers and vibrates, thereby releasing the pollen. Of the observed visitors performing buzz visits, *Pachymelus limbatus* seems to be of appropriate size to be the most effective pollinator. *Amegilla antimena* was only once seen buzzing, and seemed too small for effectively touching the stigma when turning around on the hairball of the flower, and is therefore probably only a suboptimal visitor. *P. micrelephas* on the other hand is big enough to effectively pollinate, but had very few visits overall. It is therefore likely only a secondary pollinator. Further studies and experiments are needed to disclose the dependence on the different species of bees as pollinators and whether the plant is, for example, self-incompatible. This study constitutes a step towards increasing the basic knowledge and reproductive biology of this colourful rain forest plant species.

## Acknowledgements

## References

Birkinshaw C, Lowry P, Aronson J, Lehavana A, Ludovic R, Andrianjafy M, Rakotoarivony F, Rasolofomirino J-J. 2009a. *RNC initiatives of the Missouri Botanical Garden – Madagascar*. Restoring Natural Capital.

Birkinshaw C, Andrianjafy M, Rasolofonirina J-J. 2009b. Survival and growth of seedlings of 19 native tree and shrub species planted in degraded forest as part of a forest restoration project in Madagascar's highlands. *Madagascar Conservation & Development,* **4**: 128-131.

Birkinshaw C, Raharimampionona J. 2014. *Wild fire at the Ankafobe forest: reserve for Shizolaena tampoketsana*. Missouri Botanical Garden, Madagascar Research and Conservation Program.

Buchmann S. L, Hurley J. P. 1978. A biophysical model for buzz pollination in angiosperms. *Journal of Theoretical Biology*, **72**: 639-657.

Buchmann S. L. 1983. Buzz pollination in Angiosperms. In: Jones CE, Little RJ (eds.). *Handbook of Experimental Pollination Biology*, pp. 73-112. Van Nostrand Reinhold Company Inc, New York.

Cornet A. 1974. *Essai de Cartographie Bioclimatique à Madagascar*. Notice Explicative N° 55. ORSTOM, Paris.

Du Puy D. J, Moat J. F. 1998. Vegetation mapping and classification in Madagascar (using GIS): implications and recommendations for the conservation of biodiversity. In: Huxley C. R, Cutler D. F (eds.). *Chorology, taxonomy and ecology of the African and Madagascan floras*. Royal Botanic Gardens, Kew.

Faden R. B. 1983. Phytogeography of African Commelinaceae. *Bothalia*, **4**: 553-557.

Faden R. B. 1992. Floral attraction and floral hairs in the Commelinaceae. *Annals of the Missouri Botanical Garden,* **79**: 46-52.

Faden, R. B. 1998. Commelinaceae. In: Kubitzki, K (eds.). *The families and genera of vascular plants 4*, pp. 109–128. Springer, Berlin.

Faden, R. B. 2012. *Flora of tropical east Africa: Commelinaceae*. Kew Publishing, Royal Botanic Gardens, Kew.

Lunau K. 2006. Stamens and mimic stamens as components of floral colour patterns. *Botanische Jahrbücher fur Systematik* **127**: 13-41.

Moat J, Smith P. 2007. *Atlas of the vegetation of Madagascar*. Kew Publishing, Royal Botanic Gardens, Kew.

Myers N, Mittermeier R. A, Mittermeier C. G, da Fonseca G. A. B, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature*, **403**: 853-858.

Pauly A, Brooks R. W, Nilsson L. A, Pesenko Y. A, Eardley C. D, Terzo M, Griswold T, Schwarz M, Patiny S, Munzinger J, Barbier Y. 2001. *Hymenoptera Apoidea De Madagascar et des îles voisines*. Musee Royal de L'Afrique Centrale. Tervuren, Belgique. Annales Sciences Zoologiques.

Perrier de la Bâthie, H. 1938. 37° Famille – Commélinacées. In: Humbert H (ed.). *Flore de Madagascar (plantes vasculaires)*, pp. 1–46. Imprimerie Officielle, Antananarivo.

Rakotoarinivo M, Dransfield J. 2012. *Dypsis decipiens*. The IUCN Red List of Threatened Species. Version 2014.3. WWW-document: http://www.iucnredlist.org/details/38532/0. Downloaded on 2015-08-14.

Ratsirarson J, Goodman SM. 2000. Généralités sur la forêt d'Ambohitantely. In: Monographie de la Forêt d'Ambohitantely. Ratsirarson J, Goodman SM (eds.), pp 5-9. *Recherches pour le Développement. Série Sciences Biologiques. Centre d'Information et de Documentation Scientifique et Technique* N 16, Antananarivo.

Simpson B. B, Neff J. L, Dieringer G. 1986. Reproductive biology of Tinantia anomala (Commelinaceae). *Bulletin of the Torrey Botanical Club*, **113**: 149-158.

Scogin R. 1983. Visible pigments and pollinators. In: Jones CE, Little RJ (eds.). *Handbook of Experimental Pollination Biology*, pp. 160-172. Van Nostrand Reinhold Company Inc, New York.