Initial Observations on the Pollination of *Corybas* (Orchidaceae) by Fungus-gnats (Diptera: Sciaroidea)

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Abstract

Pollinating agents of 5 Victorian *Corybas* species were studied by us in detail and it was discovered that they attracted fungus-gnat taxa of the Mycetophilidae family with a food-rewarding scent, were strictly species-specific and sexually discriminative in vector selection. A preliminary insight to early stages of the fungus-gnat taxa is also included. All information provided is based exclusively on our rigorous observations.

Introduction

*Corybas* species, also known as helmet-orchids, usually form clonal colonies (Fig 1), often hidden among grasses, mosses or leaf litter. Their flowers are small, inconspicuous, and sit low on a green, rounded to heart-shaped procumbent leaf (Fig. 3). Their ground-hugging habit made observations of the attracted fungus-gnats very difficult, and with the column completely obscured by the dorsal sepal it is impossible to see the pollination process inside the flower, whilst opportunities to visit sites were limited by the seasonally wet weather conditions.

Victoria’s terrestrial orchids that flower during the colder seasons rely on diptera members as their pollinating agents, and each of the *Corybas* taxa attracted a specific Mycetophilidae fungus-gnat. Due to their evasive nature, you had to be ready and in position near some flowers to photograph them, either on your knees or by laying prostrate on the ground, but they were more approachable when preoccupied during courtship or copulation.

The general behaviour may differ between genera of the vector species, but the pollinating procedure was fundamentally the same for all. They were attracted by an airborne scent and were observed homing in on flowers, often returning to the same one several times. Certain flowers in large colonies were most popular over several days and both sexes were observed feeding on the boss, which suggests a food-related attraction. Virtually nothing was known about the *Corybas* pollinators and primary literature to date only offered hypotheses. Based on our findings, the persisting statement in literature that *Corybas* species attract fungus-gnats as putative brood-sites is incorrect for the taxa in Victoria. No evidence of ovipositing in flowers was found. Females feeding looked gravid and were presumed to be unfertilised. All individuals looked fresh with undamaged wings and it was apparent they had recently hatched.

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Fig. 1 *Corybas fimbriatus*. A clonal colony and flowers that look more like woody fruit fallen out of the trees, randomly scattered over the ground.

Fig. 2 *Corybas aconitiflorus*. Typical habitat in eucalypt forest, often amongst similar sized gum-nuts (~14 mm).
**Flower Mimicry?**

It is commonly stated that Corybas flowers mimic or resemble fruiting fungi or toadstools, but in Victoria their cryptic appearance relates to habitat structures such as voids in the leaf litter (Fig. 2), and not any Basidiomycetes. When searching for Corybas species, the leaves are usually noticed first. The obvious bright green leaves, that look vivid pink under UV light (Fig. 3), may function as a visual cue and play a role with some of the attracted fungus-gnats that fly in low light. However, it seems more likely this colour is the most efficient for photosynthesis as many Corybas taxa grow under shrubs in dark shaded habitats, or are partially covered by leaf litter. Flowers of most taxa are generally dark in colour, but the more open types may show a reddish or white boss in relation to sun-exposure and seems to function as a form of temperature regulation.

**Pollinating agents**

The Corybas taxa observed were found to attract fungus-gnats of the Mycetophilidae family, and only of the genus Mycetophila (Fig. 4) or Phthinia. Most of their members were seen flying in low-light, in partly to heavily shaded habitats or late in the day. Corybas taxa were found to be species-specific, but unlike sexually deceptive orchids, both sexes are involved. The scent is presumed to be food-related, and it was found that a reward is present. Only one gender performs as the vector because of sexual dimorphism, being different in size or shape. With the various Corybas species, flowers have evolved to favour the smallest gender as their pollinating agent, which in the Mycetophila genus were males and in the Phthinia genus were females. Females were found to collect the pollinarium from Corybas flowers with a more accessible column, whilst the males were able to collect pollen from flowers in which inverted labellum flanges formed a more restrictive tubular passage to the column’s food-reward, possibly excluding the females.

During suitable weather conditions, when the pollen and stigma are ripe, the flowers produce a scented secretion to attract pollinating agents. A thin layer forms on the boss, which glitters when illuminated, and this leads to the main food reward, the more concentrated viscous liquid on the basal mound of the column (Fig. 5). Feeding was observed by both sexes in the flowers of Corybas fimbriatus, in which the flowers lean back, are more open and the labellum boss is clearly visible. The chemical make up of the secretions is unknown, and as each of the Corybas taxa only attract a single fungus-gnat species, they are presumed to relate to unique and specific fungal compounds.
Food-rewarding winter-orchids
The Corybas genus is closely allied to a small group that also bloom during the coldest part of the year, which includes Acianthus and Cyrtostylis. With Corybas flowers the column is completely obscured by the dorsal-sepal, but with the others it is fully exposed. Vectors could not be observed once inside the Corybas flowers, but we can speculate on the pollination procedure with confidence from the feeding observations made on their close relatives and the structures of the columns. When ready for pollination, flowers of Acianthus and Cyrtostylis have fundamentally the same strategy of enticing a potential vector feeding on the labellum towards the column along a trail formed by the secretions (Fig. 6). In Corybas flowers secretions form on the boss, but the actual reward is present as a thick liquid on the mound below the stigma (Fig. 5). Examination of a column mound in Corybas aconitiflorus was reported to secrete nectar (Coleman, 1931), but the secretions are not nectariferous and probably replicate fungus like compounds.

Sexual dimorphism in fungus-gnats
Differences between a male and female fungus-gnat of a taxon are not always obvious, but on the other hand can vary to such an extent that they appear to represent two distinct species. With such gender-forms they were usually linked by observation or when found in copulation. Sexual dimorphism is often dramatic in Sciaridae, and that the different forms attracted to Acianthus pusillus (Fig. 7) belonged to the same taxon was determined from observations, as males were commonly in pursuit of females.

In Acianthus and Cyrtostylis the distance between the labellum and anther (feeding platform and pollinia) evolved to favour the larger stocky female in order collect the pollinia (Fig. 7). Both sexes were seen feeding on the flowers at the same time, but the males have a low thorax profile and do not contact the anther. With Acianthus pusillus the primary pollinator was of the family Sciaridae, and about 10 females were observed with pollinia attached on the thorax. The Cyrtostylis reniformis vector was of the family Mycetophilidae, genus Sciophila, but only one female was observed with pollinia on the thorax.

In Mycetophilidae genera associated with Corybas species the gender differences were primarily in the body sizes. In Mycetophila the males were smallest and in Phthinia it was the females. In Phthinia the antennae lengths differed considerably (Fig. 8), with females measuring 3.5 times the eye-height and in males 5 times, whilst the eye was slightly larger in males. Antennae in the Mycetophila males were not noticeably longer than those of females. Morphological differences in abdominal shape or antennae length were usually too difficult to use for determining the gender from images.
**Corybas pollination**

As part of our research on Victoria’s terrestrial orchid pollinators, *Corybas* species were checked regularly for vectors or evidence of their activities. Fruit-setting was rarely noticed in the majority of locations and only found in small numbers when looked for in the *Corybas* taxa where vectors were observed, but swollen ovaries are not obvious until the capsules are elevated by stem elongation prior to seed dispersal (Fig. 9), almost two months after pollination. Only in *C. despectans* (Fig. 10) seed pods were seen in large numbers, but through autogamy (Fig. 11).

Of the 8 Victorian *Corybas* species, 5 were observed with their pollinating agent, and each taxon attracted only a single Mycetophilidae species (Kuiter, 2016). Rigorous observations were made on these 5 taxa over multiple seasons that confirmed them to be species-specific, as well as being sexual discriminate of their vector:

*C. aconitiflorus* – vector *Phthinia* sp 3, female.
*C. diemenicus* – vector *Phthinia* sp 4, female.
*C. fimbriatus* – vector *Mycetophila* sp 8, male.
*C. incurvus* – vector *Mycetophila* sp 9, male.
*C. unguiculatus* – vector *Phthinia* sp 2, female (?)

Many populations of *Corybas diemenicus* were regularly checked for pollinators in localities near Melbourne and Wonthaggi over many seasons, but fungus-gnats were not observed on the flowers until 2017. A site was found in Mullungdung, Gippsland, where fungus-gnats were observed interacting with flowers of *C. diemenicus* for the first time. After learning about their behavioural routine, especially being active in the afternoon, further observations were conducted in the Melbourne region.

A particularly good site was found where numerous individuals could be observed interacting with the flowers and many were seen with a pollinarium attached. Seemingly unperturbed by our presence, their behaviour and the pollinating procedure could be studied in greater detail.

*Corybas unguiculatus* and their fungus-gnat vectors were only observed over two seasons. Many were seen flying out of the flowers with pollinia attached, but only one was photographed, which had landed in the grass close by. It was identified as *Phthinia* sp 2, and it was a female, but the sex of the vector needs to be confirmed for this species.

Due to location remoteness *Corybas hispidus* has not yet been observed in detail, whilst *C. fordhamii* can only be accessed after fire removes obstructions in their swamp habitats. These taxa are expected to attract very closely related fungus-gnats as those documented here.
Pollination Process in *Corybas*

The completely hidden column in *Corybas* flowers makes it impossible to view the pollination process, which has led to much speculation and hypotheses by different authors. To get an good understanding of the procedure, it took countless hours of observing the interactions of the pollinating fungus-gnat species, as well as studying the structures of the various *Corybas* flowers.

The flower construction shown here is for *Corybas diemenicus*, but fundamentally is the same for the other members of the genus. Fungus-gnats usually feed on secretions on the boss for some time, and potential vectors move in further, directed toward the column. Fluid on the basal mound (Fig. 14-D) may emit a stronger scent and is thought to provide the main reward. Unable to exit through the narrow openings at the base, it moves back to leave to flower. In retreating, the thorax collects the sticky viscid disc just above the stigma and the pollinarium becomes attached on top of the thorax, and in *C. diemenicus* just posteriorly to the highest point. To exit the flower it manoeuvres itself upwards, using the spiky legs to push its head up to turn around and climb up to the dorsal sepal, using the edges of the labellum’s inverted flanges. The column in *C. incurvus* is angled back and there is enough space above the anther for a vector to turn around and climb out, and the pollinarium is attached more anteriorly on top of the thorax. Both sexes entered the flowers of *C. incurvus*, but only the males were seen with a pollinarium. The restrictive passage of the tubular tunnel seems to prevent the larger stocky females from reaching the column.

*Corybas aconitiflorus*, *C. diemenicus* and *C. unguiculatus* attracted *Phthinia* taxa and female vectors. *C. fimbriatus* and *C. incurvus* attracted *Mycetophila* taxa and vectors were male.

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**Fig. 12** *Corybas diemenicus* with dorsal sepal lifted. The boss narrows towards the back, curving into a restrictive tubular structure leading to the column. In some species it has short but dense downward pointing hair-like structures.

**Fig. 13** *Corybas diemenicus*, dorsal sepal removed. Labellum from the back, tubular part evenly narrowing.

**Fig. 14** *Corybas diemenicus*, with dorsal sepal and parts of the labellum cut away. A Labellum forming a tubular tunnel behind the boss. B Side view of labellum, showing inverted flanges and bottom flanges, under which small openings into the flower to allow for air movement. C Back view with dorsal sepal removed also showing openings at the bottom. D Side view of column, showing the proportionally large mound below stigma, glittering from secretions.
**Corybas aconitiflorus**
Observations on this *Corybas* taxon were conducted regularly over several seasons at a few locations in Gippsland. The main site comprised colonies with good numbers of fresh flowers scattered over a large area and was visited over consecutive days, with sessions from noon for up to 5 hours. The weather conditions were mild for the time of the year (June) when vectors were observed and many other diptera insects were active, including a *Mycomya* sp., which was photographed amongst flowering *Corybas* on the ground. It showed the wing-venation perfectly, and in the assumption of it being the vector species, led to a misidentification at first (Kuiter, 2016).

*Corybas aconitiflorus* attracted *Phthinia* sp 3 and many individuals were observed, males seemingly outnumbered females, but only females were seen with a pollinaria attached (Fig. 15). A vector feeding on the basal mound of the column may contact the viscid part of the stigma with the thorax and collect the pollinia as it retreats. To leave the flower it does a upward rotation to turn around and climbs out, coming out head first. Both sexes were observed going into the flowers, but no males emerged with pollinia and were presumably too large or the wrong shape to reach the column. At least 6 females were observed leaving the flowers with pollinia dorsally attached on the thorax, flying out without stopping. On a few occasions they landed in grasses a few metres away and were attended to within seconds by males, attracted to the pheromones being released. They would either fly away whilst in copulation or disappear in the leaf litter before pictures could be taken.

The legs of fungus-gnats are not designed to walk on and in flight they function as feelers instead of the antennae. When positioned upright they use the spurs on the joints to crawl or stand on, and when upside down use the hooked tips to hang on. Abdominal probing by fungus-gnats on *Corybas* flowers was regularly observed, which could be interpreted as egg-laying, but it appears to be an impulsive habit and used in positioning itself. They were also probing with their abdomen as they walked on the leaves and on adjacent soil, and this behaviour was seen for both sexes. It was apparent that feeding females had recently hatched, probably earlier that day, and had not yet mated. They were observed on a number of flowers for a considerable time on each, and when finished, feeding mating took place soon after. Eggs are presumed to be laid only in suitable habitats for larvae of *Phthinia* members, such as moist rotting logs on the ground, rich with associated saprophytic fungi.

![Fig. 15](image1.png) A female *Phthinia* sp 3, emerged from *Corybas aconitiflorus* with pollinaria. It was positioned over the base of a wing, which made flying difficult.

![Fig. 16](image2.png) A female *Phthinia* sp 3 on *Corybas aconitiflorus*. Upper: licking on hood which may have some secretions. Lower: The probing with the abdominal tip may suggests ovipositing, but this behaviour seems to be only a habit or for positioning itself.
Corybas diemenicus

Vectors of this Corybas species were found to be the most illusive of all the helmet-orchids that were monitored. Numerous colonies were checked over many seasons in regions of southeastern Victoria and no pollinating agents were found until 2017. The first fungus-gnats observed interacting with flowers was at a newly discovered site in Gippsland, where large colonies with numerous fresh flowers were found in coastal eucalypt forest in the shade of dense shrubs. They were growing in seasonally moist sandy soil, which was predominately covered with mosses and leaf litter.

Once familiar with the Corybas fungus-gnat routine regular visits were made to the same site. A female with pollinarium attached was seen feeding on the boss of several flowers, spending some minutes on each one. The females were seen probing with their abdomen (Fig. 18), but this is a habit shared by many other fungus-gnat taxa. Males appeared to be patrolling a flowering colony, waiting for females to become sexually active and then approach them with a courtship routine. As found with the other Corybas vectors, the fungus-gnats copulated after feeding, and several pairs were observed mating later in the day. The males of this Phthinia species were considerably larger, but this form of sexual dimorphism appears to be typical for this genus. With restricted access to the column, it seems that only the smaller females can reach the column or have the right morphology to collect a pollinarium. The female’s thorax appears to be more elevated and slightly shorter (Fig 17-inset) than in the larger male. Males can also be identified by the much longer antennae Fig. 22).

Fig. 17 Corybas diemenicus and its pollinating agent, a female Phthinia sp 4, with a pollinarium collected from another flower. Body length is about 3–4 mm. Inset Female. showing the high profile of the thorax.

Fig. 18 Phthinia sp 4, vector on Corybas diemenicus.
Fig. 19 Phthinia sp 4, dorsal view, showing wing-venation and colouration of wings, thorax and abdomen, similar to sp 3, vector of Corybas aconitiflorus.

The fungus-gnat taxon was identified as a member of the genus Phthinia using the wing-venation and is referred to as sp 4. The larvae of this genus were found to be living in decaying logs on the ground in moist habitats. They feed on saprophytic fungi, and two different species of Phthinia were hatched from larvae on some pieces of bark collected in the field.

Discussion

It had taken multiple seasons to discover a site where the pollinating agent of Corybas diemenicus was present and could be studied. After discovering the conditions and the time of the day when the fungus-gnats would be interacting with the flowers, our first observations on this vector species were made over many days during 5–6 hours sessions. To fully comprehend the fungus-gnat behaviour and pollinating procedure, it was essential to repeatedly conduct observations over such long sessions for the preparation of the initial documentation.

At the Mullungdung site, Corybas diemenicus flower visitation varied from day to day, but the flying times of the fungus-gnats were consistent for the attracted taxon. Their activities started about 1:00 pm, usually peaking for about one hour and continued until late on some days. The weather conditions ranged from unseasonally warm to days with frosty mornings, but the fungus-gnats were most abundant on the warmer days. On the cooler days only a few fungus-gnats were observed, and usually showed less interest in the flowers. They were possibly not hatching during the very cold periods, and the flowers may only emit a weak scent, and probably have no reward present.
**Corybas diemenicus var. Kallista**

Populations growing on the trunks of tree-ferns in the Dandenong Ranges were checked many times over several seasons for pollinators, and finally in 2017 fungus-gnats were observed, including many vectors with pollinia attached. On the first day, about 10 individuals with pollinia were seen, some may have been observed twice, but all were female. The taxon was identified as *Phthinia* sp 4, the same species as the one found on the colonies of *Corybas diemenicus* in Gippsland.

No activity was seen until after 1:00 pm. Females were first noticed, as they were flying with pollinia on their thorax and this made them more obvious. They were observed feeding on the boss of many flowers, going inside and out of sight for several minutes in each one, totalling a period of at least 30 minutes. Over 6 individuals were seen coming back out of the narrow tubular part of the labellum behind the boss. In order to collect the pollinarium a vector has to go down the tubular section for the thorax to contact the viscid part above the stigma, obtaining the glue which collects the pollinia as it retreats to leave the flower. It turns around by an upward rotation in the more spaced section of the tubular part above the column (see page 5) and climbs out, using the edges of the inverted labellum flanges to reach the dorsal sepal. Individuals were observed flying out upside down (Fig. 26) from a hanging position in the flower.

The female vectors with a pollinarium attached were concentrated on in order to learn the procedure, and consequently very few males were photographed (Fig. 24), but most importantly included one in copulation.

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**Fig. 23** Corybas *diemenicus* and a female *Phthinia* sp 4, with a pollinarium from another flower, flying in to feed.

**Fig. 24** Male *Phthinia* sp 4. Sex was determined by the long antennae, but it also has the slender anterior abdomen.

**Fig. 25** Corybas *diemenicus* with a female *Phthinia* sp 4. Probing with abdomen seems to be to position itself.

**Fig. 26** *Phthinia* sp 4. Flying away after exiting, and still upside down as it has just let-go from the dorsal sepal.
Fig. 27 Phthinia sp 4, a close-up showing the pollinarium is well secured on the upper-posterior part of the thorax. After about 30 minutes feeding, she was probably emitting pheromones and waited for about 10 minutes for a male to arrive.

Discussion

The site is situated in a deep dark gully, dominated by Mountain Ash forest, with an understory of large tree ferns, on which due to lyrebird disturbances the orchids are relegated to grow. It was apparent that the fungus-gnats on the day were newly hatched, had not yet copulated and only entered the flowers to feed. They were observed licking secretions on the boss of many flowers, reminiscent of feeding habits witnessed on the related winter orchids.

After feeding, the females flew to a nearby spot to attract males and copulate, waiting while emitting pheromones. In one instance a male took about 10 minute to arrive (Fig. 27 & 28). Ovipositing would follow some time later in the day, probably in the decaying logs on the ground that are broken down by saprophytic fungi. Adult stages of most of the fungus-gnat taxa are thought to be to be short-lived, usually lasting only a few days, probably until egg laying is completed.

Their emergence from the pupa stage appears to be related to atmospheric conditions. On the day they were seen flying in large numbers, it was mild and sunny after several cold and wet days. The next day on a similar, but more windy day, none showed at all. Several more vectors were observed a week later on another calm sunny day after 1:30 pm.

Flower colour is typically very dark in the heavily shaded habitats, and even on a sunny day they were very difficult to focus on with the camera. To be able to capture images of a vector coming out of the tubular tunnel a torch light was needed to see it re-appear, before typically flying straight out.

Fig. 28 Phthinia sp 4, feeding on the boss of Corybas diemenicus for many minutes, and after feeding emitting pheromones to attract a male for copulation.
Corybas fimbriatus

This taxon attracted Mycetophila sp 8 and at least 10 individual vectors with a pollinarium attached were observed, and were identified as males. Many females were observed feeding on the boss (Fig. 30/31), with some entering the flower, often going out of sight for several minutes, but none came out with a pollinarium. Females may be too stocky to reach the column through the restrictive tubular part of the labellum, or their thorax morphology is not compatible for collecting a pollinarium.

Observations were conducted at sites from about noon until dark and it took many sessions to find the vectors. The first fungus-gnats with a pollinarium on their thorax were seen flying over a colony late in the afternoon, but it seemed impossible to get images. Their flying was rapid and erratic, whilst it was getting much too dark to see, and after landing they would quickly disappear into a flower, but a female was photographed when it landed on the boss in front of the lens. It spent some time feeding and was identified to a genus based on the wing venation (Fig. 33). Subsequently more identifiable images were taken at different localities, and all represented the same species. It seems this taxon primarily flies in low light conditions, but observations were made on mild, partly cloudy or sunny days. It’s likely that they are active on overcast and rainy days as well, and may prefer heavily shaded bush habitats.

Vectors were mostly observed on clonal colonies with large flower numbers near decaying logs in wet environments. Logs in an advanced state of decay are primary breeding sites for fungus-gnat larvae, where they feed on fungal hyphae. Larvae of taxa that are pollinating agents of many terrestrial orchids were found under bark, including some of the Corybas vectors.
**Fig. 33** Female *Mycetophila* sp 8 feeding on the boss of *Corybas fimbriatus*, clearly showing the wing-venation, which identifies the genus in the Mycetophilidae family.

**Fig. 34** Male *Mycetophila* sp 8 emerged from the flower of *Corybas fimbriatus* with pollinarium. It kept flying on the colony for some time, probably searching for female.

**Fig. 35** *Corybas fimbriatus*. Showing typical integral structure of the column, which is finely tuned to the morphology of the fungus-gnat, and works only with the male gender.

1/ pollinia pair that looks fused into one. 2/ viscid part at the top of the stigma. 3/ stigma, a disc with a sticky surface. 4/ basal mound that secretes the food-reward when the flower is ready and conditions are suited for pollination.

In principal the design is virtually identical in the members of *Corybas* that were studied, but in this species the features seemed to be more defined.
**Corybas incurvus**

Observations were conducted over several seasons at many sites in various regions in Victoria, but vectors were observed at only a few sites. *Corybas incurvus* attracted *Mycetophila* sp 9, and at one site about 6 males were seen with a pollinarium attached. At this site, both sexes of this Mycetophilidae taxon were observed in good numbers, and often males were seen following a female. Copulating pairs (Fig. 37) were photographed over different seasons, and the male was confirmed as being the vector.

In *Corybas incurvus* the column is angled backwards and there is a space directly above in which a vector can manoeuvre up and climb out the flower. When the viscid glue is obtained, the pollinarium becomes attached to the frontal curve on top of the thorax. In *Mycetophila* the male is considerably smaller than the female, but otherwise sexual dimorphism is not obvious. Females are probably too large or stocky (Fig. 39) to come in contact with the stigma or only the males have the correct thorax morphology to obtain the pollinarium.

At 3 other sites this fungus-gnat was only seen in small numbers, despite many hours of observation. At a Mornington Peninsula site, fungus-gnats were only observed on a rainy day with a male on a flower at 3:00 pm (Fig. 42), and another about 30 minutes later. The colony was checked over 5 days and no others were seen. None were observed on a colony checked near Belgrave, but some weeks later some swollen ovaries indicated that pollination had taken place. At Mullungdung no activities were seen for many days, until a male with a pollinarium was found and with females arriving, multiple courtships and copulations were witnessed.
Courtship behaviour
The male shown here with the pollinarian attached was observed from about 1:00 pm for 3 hours on a single colony. When a female arrived (Fig. 38) the male was witnessed performing a courtship routine on or near a flower, comprising jump-like moves and short bursts of rapid wing-beats. The female seemed submissive, approaching the male to a spot nearby where copulation took place. Soon after she would return to the flower and feed on the boss. Another female arrived 21 minutes later and again the courtship and copulation took place by the same male. Courtship behaviour had been observed the season before, but due to the presence of several males was confusing, and getting images was the main priority at the time. The males were seen also performing with rapid wing flapping and body motions (Fig. 40). Courtship behaviour in several genera of the Mycetophilidae family is known from the northern hemisphere, but it was not observed in Phthinia taxa on the Corybas.

Note 1
A presumed fungus-gnat pollinator was reported on this species, then named as Corybas diemenicus, from Mount Morton, near Belgrave (Jones, 1970). It was correctly identified at the time, but examination of the type specimens in Kew showed the mistake and led to the subsequent naming of C. incurvus as a distinct species in 1988 (Jones, pers. comm.). The vector showing attachment of pollinarian was found dead on a flower, trapped by some spider web. The fungus-gnat was illustrated in a very detailed drawing and looked identical to the male Mycetophila sp 9.
**Corybas unguiculatus**

Populations in the Melbourne regions of this taxon, commonly known as the Small Helmet-orchid or Pelican-orchid, have rapidly declined over the last 2 decades. One population in the Langwarrin Flora & Fauna Reserve historically produced seed capsules in large numbers, but a fire completely destroyed it before studies could be done. Another population, comprising about 30 to 40 flowers, was discovered in a different part of the reserve in 2014, and has been monitored since, but fruit rarely sets.

The fungus-gnat species attracted to this Corybas was first observed in 2016 at a single site in mature coastal tea-tree habitat, where previously seed pods had been seen. On certain days they were seen flying in good numbers, but many visits were required to make the observations and it took countless hours to get sharp images of these very evasive, erratic and quick flying fungus-gnats. Most were seen in the afternoon between 1:00 and 5:00 pm. The taxon was identified as a member of the Mycetophilidae genus *Phthinia*. Several vectors with a pollinarium attached on the thorax were seen flying out of the flowers, but no opportunity was presented to get a photograph. Based on the many images taken, it was apparent that both sexes were attracted, but gender of the vector was not determined during that season.

The next season only a very few fungus-gnats were seen at the same coastal site and it seemed that none of the flowers were setting fruit. Due to unseasonably dry weather, flowering was poor and the habitat was drying out with flowers aborting at the site.

Searching a hinterland eucalypt forest resulted in locating some excellent populations to work with, and with flowers already setting fruit it was clearly a good site to continue our observations. The site was visited on many days and fungus-gnats were regularly seen in good numbers on the flowers, and at this locality they were also active from early in the afternoon. The more open grounds with better light made it easier to get images, and finally a vector with pollinarium attached was captured on camera. It was determined to be a female and most of the individuals seen going on the flowers appeared this gender. Females were smaller than males, but more observations need to be made to confirm the gender of this orchid’s vector and also if the size disparity is a factor, but it would be consistent with other *Phthinia* members.

**Discussion**

Fungus-gnats associated with *Corybas unguiculatus* were found to be difficult to locate and observe. At the same time the fungus-gnats were flying in Gippsland, the Langwarrin site was visited on many days in the afternoon, but failed to see pollinator action. During
observations over about 5 weeks at this site, only 2 out of the large number of flowers were setting fruit, but it indicated that the vector was present in the area.

*Corybas unguiculatus* needs to be observed over additional seasons to learn more about the pollination process, the vector activities and their occurrences. Gender of the vector needs to be confirmed and the behaviour of the fungus-gnat taxon further studied. Based on our rigorous work done with fungus-gnats and their association with the *Pterostylis* genus, the greenhoods and rustyhoods, it seems that many of the diptera members have declined in numbers. They are threatened due to habitat alterations, especially from inappropriate fires that dry out their habitats, destroying the larval fungal food-source, as well as from the overuse of pesticides and other chemicals.

*Fig. 47* Male *Phthinia* sp 2 has longer antennae and is larger than the female, and shown here is the typical more slender shape in the anterior sections of the abdomen.

*Fig. 48* *Phthinia* sp 2, female, attracted to a flower of *Corybas unguiculatus*.

*Fig. 49* Female *Phthinia* sp 2 on an exceptionally large flower. Opening about 5 mm in width.

*Fig. 50* Female *Phthinia* sp 2 about to enter the flower. The opening was measured in several fresh flowers in one of the colonies at the site, and ranged from about 4 to 4.5 mm.
**Summary**

It requires many decades of equally in-depth studies to comprehend both the terrestrial orchid taxa’s flowering regimes and the irregular occurrences of their pollinating agents. The *Corybas* species and their diptera fungus-gnat vectors rely on certain sensitive wet habitats, of which stability is essential for their long term perpetuation. Regular observations were limited in this often difficult habitat by the seasonally short days and the wet weather conditions. Learning about the pollination procedures required many hours of observations over their flowering periods, but to be able to document the complete sequence of events it took several seasons. To observe the vectors interacting with the orchid flowers, one has to be able to predict when this is likely to occur, which requires a good knowledge of the insects. To understand the symbiotic relationship of *Corybas* and fungus-gnats in Victoria, it was necessary to learn about the life-cycle of the fungus-gnats, especially where larvae lived.

To record the behaviour of the pollinating agents and to obtain details of their morphology, required to identify an attracted species to a genus level and to determine the gender, a very large number of images were taken. Our findings on the pollinating agents on 5 out of the 8 Victorian *Corybas* taxa, provides a good frame work to build on, investigate more species and to elaborate in much more detail on their dipteran symbiotic relationships.

**Vector routine**

Activity of the fungus-gnats attracted to *Corybas* flowers was generally confined to early and mid afternoon, suggesting emergence from their pupal stage earlier in the day. It was apparent that both sexes feed shortly after emerging, and as soon as they were able to fly. They are presumed to require nourishment prior to copulation or ovipositing, especially for egg development in females. The feeding routine of females was usually over periods in excess of 30 minutes by going from one flower to the next, spending a few minutes in each one. As the females seemed to take longer in feeding, anxious males would be ready for copulation, and this was observed as soon as females began emitting their sex-pheromones. With the fungus-gnats attracted to *Acianthus pusilla*, copulation was observed after feeding as well, and the females observed on labella generally looked heavily gravid.

**Eggs on *Corybas* flowers**

In light of the hypothesis that *Corybas* taxa attracting female fungus-gnats as a brood-site, commonly stated in publications, we made a special effort to investigate if this was true. We found no evidence of ovipositing in the flowers by fungus-gnats, nor convincing proof in support of the hypothesis in literature. Eggs were found on some *Corybas* flowers, but these proved to be from the Collembola order and not from Diptera. The jelly-bean shaped eggs from a Sciaridae member (Fig. 51) were
measured, 0.22 mm long, but sizes and shapes would vary in relation to different taxa of the Mycetophilidae family. A few Arthropoda adults were observed on some Corybas taxa and one individual was found to lay eggs in a flower (Fig. 52). Some tiny first instar hatchlings, about 1mm long were sometimes found on the boss, and may resemble small maggots. They are white and appeared to be immobile, but were ruled out being of the diptera order as legs developed after moulting in the next instar (Fig 52-inset).

**Looking for Mycetophilidae larvae**

Virtually nothing seemed to be known about larval stages of the Mycetophilidae species in Australia, other than them being associated with fungi (Colless, 1970). In the northern hemisphere the fungus-gnat families are well studied, and information found about early stages of closely related species on http://sciaroidea/info and for the Nearctic zones in the Manual of Nearctic Diptera (J.R. Vocenor, 1981), revealed that most of the species we needed to investigate lived in rotting logs and fed on fungal mycelia.

Some Corybas taxa colonies may occur for many years adjacent to rotting logs, and in the shared micro-habitats their associated fungus-gnats would not have to fly far to the flowers after emerging from their pupal stage. The search for larvae (Fig. 53/54) was conducted within the orchid’s habitat during the flowering period, and were readily found in decomposing logs on the ground that contained fungal mycelia.

**Hatching of fungus-gnats**

Small pieces of bark, that harboured larvae, were taken and placed in an aquarium with a glass lid. The larvae that were visible ranged from about 15–25 mm in length and thought to comprise at least three different species. Further specimens were collected a few weeks later and the number of species rapidly grew, and as the images show these wet habitats support a great diversity of diptera taxa. From the orchids perspective this shows how important it is not to interfere with habitats, especially with inappropriate fire regimes, that causes the drying out and consequent loss of habitats for the diptera larval stages of pollinators, on which winter and early spring flowering orchids rely.

The first fungus-gnats, about 3 mm in body length, hatched after about two weeks and were identified as members of the family Mycetophilidae, the genus Phthinia, based on wing-venation. About 10 individuals emerged over 4 days, probably originating from the smaller larvae (Fig. 53), and were usually observed in the afternoon. Another member of the genus hatched a week later.

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Many different taxa hatched, including a species of the genus *Mycomya* (Fig. 58), which would have developed from the 25 mm larvae (Fig. 54), and members of this genus are sexually attracted pollinators of a large number of *Pterostylis* taxa. It was surprising in having so many species across several different genera hatching from a small amount of bark.

An unusual female was tentatively identified as *Paramorganiella adventurosa* (Fig. 59), as it matched the original description by Tonnoir, 1929, and also in wing-venation. This taxon has mandibles and orange spurs on the legs, and it was only known from a single male collected on Bruny Island, Tasmania.

Distinctive larvae with a black head collected at the *Acianthus pusilla* site were assumed to be members of the Sciaridae family, as many adults emerged from the collection. The female shown (Fig. 60) is thought to be of the *Trichosia* genus and appears to be the commonly observed taxon feeding on those *Acianthus* flowers (Fig. 7).

The pollinating agents of all the *Corybas* and almost every *Pterostylis* species are fungus-gnats of the Mycetophilidae family. All these orchids depend on seasonally wet undisturbed-habitats, whilst their diptera pollinator species rely on decomposing tree branches and logs on the ground for their early stages. This natural process of recycling provides nutrients back into the ecosystem, converted into the food and suitable habitats for the orchids and their pollinators.

**Literature cited**


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