

Overview of *Pterostylis* Pollination (Orchidaceae) in Victoria

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Overview of *Pterostylis* Pollination (Orchidaceae) in Victoria

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Abstract

Members of the large terrestrial orchid-genus *Pterostylis*, collectively known as greenhoods and rustyhoods, attracted only insects of the order Diptera, the flies, as their principal or primary vectors. With few exceptions, the pollinator species were part of the superfamily Sciaroidea: infraorder Bibionomorpha: families: Mycetophilidae and Sciaridae, that are commonly known as fungus-gnats and dark-winged fungus-gnats respectively.

Introduction

All information provided is based exclusively on our extensive observations. Only species that proved to be convincing pollinators were included as principal or primary vectors. Identification was limited to the genus level, primarily based on their wing-venation, using European taxonomy (sciaroidea.info). Taxa identified as vectors were assigned a number within each genus for reference purposes. Observations were made on sites during conditions when insects were expected to be active, and by watching a colony, or a small number of plants within view from one point. Many hours were devoted to common orchids to understand the insect relationships and anticipate pollinator activity. E.g. with *Pterostylis nutans* it took over 20 hours in the first season to learn about pollination, the vector and to photograph the procedure. Many greenhood taxa were studied over consecutive seasons and this revealed that most of the rarely seen pollinator species may only be abundant for a very short period of a few days, or even absent, in any given season.

Pollination was witnessed in about fifty *Pterostylis* taxa and images were taken of all the vectors. The pollinators were comprehensively published in a book (Kuitert, 2016), and with this paper our aim is to highlight the relationships between the orchids and their respective vectors, methods of attraction, and the correlation between the Victorian *Pterostylis* clades and insect genera.

Attraction

The majority of the fungus-gnats involved in the pollination of *Pterostylis* were identified as males in being the principal vector and were attracted to the orchids by sexual deception. The flowers emit a wind-borne scent (kairomones), presumably a mimic of a female fungus-gnat's sex pheromones. Greenhoods in which the labellum is mostly hidden inside the galea, a strong scent is emitted from the osmophoric tips of long lateral-sepals. The base of the labellum has an appendage with a "fluffy top", often referred to as the lure, which may produce an

additional scent. Several small greenhoods taxa, that lack long lateral-sepals, apparently produce a food-suggesting scent, which is probably emitted primarily from this lure. Taxa with a fully exposed labellum emit kairomones, that are produced most strongly from the basal mound.

The principal Greenhood pollinators

All medium to large hooded taxa were pollinated by Mycetophilidae fungus-gnats. The vectors that were observed belonged to six genera: *Allodia*, *Leia*, *Mycetophila*, *Mycomyza*, *Orfelia* and *Phthiria*. With the *Pterostylis* vectors the pollinia were attached to the highest part of the thorax (Fig. 1). Most pollinators were members of the following two genera: *Mycomyza* and *Mycetophila*.

Genus *Mycomyza*

The genus *Mycomyza* (Fig. 2) was found to have the largest number of principal vectors, with 12 species identified on 16 different greenhoods, 6 of which were tall-greenhoods. *Pterostylis* taxa attracting *Mycomyza* were found to be strictly species-specific, but historically allopatric greenhoods sharing the same vector may now occur sympatric because of habitat alterations, and hybridise. The taxa: *P. acuminata*, *P. cucullata*, *P. falcata*, *P. nutans*, and highland taxon *P. oreophila*, were all pollinated by *Mycomyza* sp 2 (Fig. 3). Two hybrid forms are well known, and were originally named as *P. aenigma* and *P. ingens*. *Mycomyza* sp. 2 is apparently a very widespread taxon, seasonally common in the coastal regions from about May, and was observed in the highlands in December.

Hybrids between sympatric sibling *Pterostylis* taxa attracting different *Mycomyza* vectors are extremely rare, even when growing next to each other. Geographically well separated populations of a greenhood taxon may have different vectors, but usually are closely related and allopatric. Observing two different *Mycomyza* species as vectors of *P.*



Fig. 1 Nutans Fungus-gnat *Mycomya* sp 2. It came out with fresh pollinia on the highest part of the thorax. An eroded pair under it came from another flower.



Fig. 2 Wing venation of *Mycomya*.



Fig. 3 *Mycomya* sp 2, about 5 mm in body length. The genus is recognised by distinct wing-venation (Fig. 2) and the species by thorax markings and body colour.



Fig. 4 Wing venation of *Mycetophila*.

melagramma at the same sites in well separated areas, was an exception. It's likely that the insects were historically allopatric and sympatry had occurred with habitat alterations. In coastal regions the *Mycomya* fungus-gnats fly most of the day, and the males were often seen entering flowers that just became exposed to direct sunlight.

Genus *Mycetophila*

The next largest genus (Fig. 4) was with 9 vectors identified on 10 greenhood species. The vectors are relatively small, but they pollinate some of the larger greenhood flowers (Fig. 5). Many of the small flowered *Pterostylis* that sexually attracted *Mycetophila* vectors were not only species-specific, but were also visited by taxa of the Sciaridae family (both sexes), possibly as secondary pollinators, and may explain unexpected hybrids. Ancestral forms would have attracted pollinators with food-related methods, that may still be present in a rudimentary form in small greenhoods with osmophoric sections on lateral-sepals. Most *Mycetophila* members were active in the afternoon and some species were only seen on dusk and possibly are nocturnal as well. Only *Pterostylis alata* and *P. dolichochila* shared the same vector species.

Pterostylis tunstallii was the only tall-greenhood attracting a member of the *Mycetophila* genus, and it has a short labellum to match the small vector size. The only other tall-greenhood with a short labellum is *P. diminuta*, which currently is an exception in the genus *Pterostylis* by attracting a member of the genus *Allodia*. Tall-greenhoods with the longer labella attract the larger *Mycomya*.



Fig. 5 *Pterostylis grandiflora* with *Mycetophila* sp 3. It has large flowers, but its vector is tiny, ~3 mm. One individual outside and one emerging with pollinia.

Genus *Leia*

Members of this genus (Fig. 21) are small, about 2.5 mm in length (Fig. 6) and only 3 species were identified as vectors of *Pterostylis*. The principal vector sizes are in relation the column morphology of the species they are attracted to, as one species is the vector of the large-flowered *P. baptistii* and two species are of the small-flowered *P. atrans*, *P. fischii* and *P. pedunculata*. Latter two share the identical vector, but bloom several months apart in the same areas.

Family Sciaridae

Members of the Sciaridae (Fig. 22) were usually found to be secondary pollinators with greenhoods that have a male *Mycetophila* vector, but their attraction is food-related when involving both sexes. Of the small greenhoods with osmophoric sepals, only *Pterostylis nana* was found to sexually attract male Sciaridae members. With *P. cygnocephala* & *P. mutica* clades (Fig. 7), only males were found to be principal vectors and, while these greenhoods are species-specific, they lack osmophoric sepals. Like with the rustyhoods, kairomones are emitted from the basal labella mound. Males were observed running over the flowers, looking more like black ants, and when engaging with the labellum were usually propelled into the hood. The two vector species appeared to belong to the genus *Trichosia*. Both were black, one completely and the other with brownish legs and were a very small taxa, with a body length of just under 3 mm.

Greenhood-pollinator behaviour

Flowers of *Pterostylis* taxa may look very different in their structure (see page 10), but reproductive mechanisms, trapping of vectors and the pollinia delivering escape routes (Fig. 9), are the same. Numerous individuals were observed entering the Nodding Greenhood, *Pterostylis nutans*, and the ones collecting pollinia usually came out after about 5–12 minutes. When an insect attempts copulation with the labellum and causes it to trigger, it may become trapped inside where the stigma is waiting to receive pollen. When entering with pollinia on the thorax from another flower, it will brush them against the stigma and deposit pollen on the sticky surface. The translucent galea section may confuse the insect and usually it takes a considerably long time to find the escape route. The pollinia may hit the stigma several times before the vector positions itself and moves into the column-wings space. As it goes through, the high thorax collects some glue from the sticky pad of the rostellum, picking up pollinia on the way out (Fig. 8). It usually flies away with them, often without stopping, but sometimes requiring a brief recovery, and some were observed flying straight to and entering another flower.



Fig. 6 *Pterostylis atrans* with *Leia* sp 2. Usually males are first attracted to the sepaline osmophores.



Fig. 7 *Pterostylis mutica* with a sexually attracted male Sciaridae sp 6 (*Trichosia*?).



Fig. 8 *Pterostylis smaragdina* var. *rubrirostrata* with *Mycomya* sp 8 coming through the column wings.

Depending on species, prevailing temperature, and flower condition, a labellum may reset within a few minutes when warm and up to about 30 minutes when cool. The longest time to reset was observed with a tall-greenhood, taking over one hour, which was a seemingly old flower. When triggered, fluid is drawn in to the strap, inflating its tissue to reset the labellum. To become sensitive again, it will take a similar time to become motile, as this depends on the same fluid to withdraw.

Some of the greenhoods have distinct osmophores on the lateral sepals and male fungus-gnats often land on them first. This was witnessed with many *Leia* individuals on *Pterostylis atrans* (Fig. 6), where they rotated around them at great speed, and only a few were seen going into the flowers. A good number of plants were found loosely scattered in a grassy area under some trees and flowers were checked for pollinia, but only a plant found bent over in the grass had its pollinia in place, and it was stood up. It didn't take long for a fungus-gnat to enter and come out with pollinia on the thorax. The vectors were numerous on the day, but not seen the next season when checking at the same site.

With the tall-greenhoods the scent is produced from the basal labellum mound, but the males often grab the synsepalum as they land (Fig. 10). It seems to put extra tension on the strap and the labellum flicks up when it moves forward and lets go of the synsepalum, usually propelling the vector into the hood. The males can be seen probing with their abdomen in the search to copulate. Tall-greenhood fungus-gnats are usually common during favourable conditions and often several individuals can be seen taking an interest in the same flower.

Dense flowering plants – No pollinators

Large dense-flowering greenhood colonies formed on the Mornington Peninsula after a decade-long drought and vectors were apparently absent, as no seed pods were found. It took about 3 years for *Pterostylis nutans* vectors to return or recover, and dense colonies no longer formed. With *P. concinna* the large dense-flowering groups were still forming and vectors seemed to have remained absent. Most populations have been wiped-out by prescribed burning, making observations difficult. Pollinators on *P. revoluta* were looked for in most parts of Victoria over different seasons and no seed pods were found in localities where the species formed these dense-flowering colonies. Pollinator absence or presence and also fire clearly effected these species in their flowering-plant density each season. Various *Pterostylis* vectors fluctuated in numbers between seasons and may be rare most of the time, but abundant with certain conditions in a season or have life cycles involving a number of years.

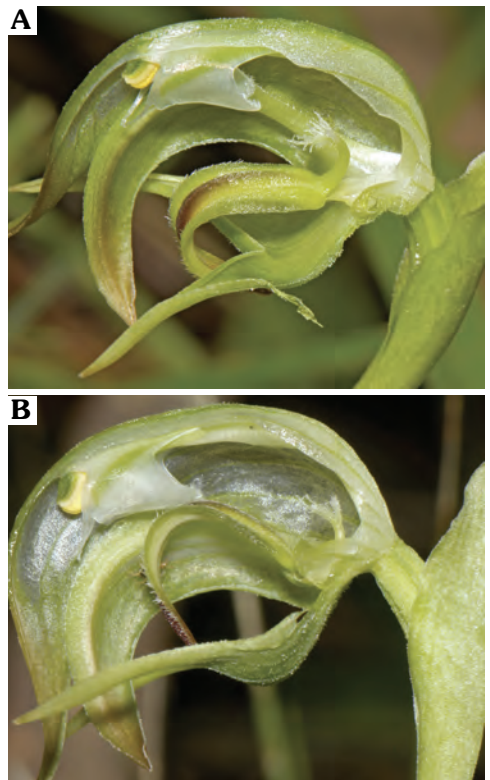


Fig. 9 *Pterostylis nutans* with side removed to show the internal trapping mechanism and winged column. **A** labellum on a short strap in set position and the lure, its fluffy appendage, at the base. An attracted vector would go on the labellum, moving towards the lure, which triggers it when sensitive, closing the entrance and trapping the fungus-gnat. **B** The escape route is through the opening between the column-wings where its thorax hits the viscidium and collects the yellow pollinia on the way out, usually a pair.



Fig. 10 *Pterostylis smaragdyna* var. *rubrirostrata* with *Mycomya* sp 8, moving up the labellum

Rustyhood pollinators

Rustyhoods are highly advanced in evolutionary development and have become very specialised, adapting to harsh inland environments and dry rocky-gravel habitats, forming a distinct *Pterostylis* clade. All species of the rustyhood group sexually attracted fungus-gnat males of the genus *Orfelia* (Fig. 11). Fungus-gnats in remote arid regions may rarely have a good season, as their larval stages rely heavily on the limited moist habitats available.

Five currently recognised rustyhood species were found to be pollinated by the same vector, *Orfelia* sp. 1 (Fig. 12). Taxa having the same pollinator would have derived from relatively recent ancestors and evolved into many different local varieties, adapting to environmental conditions, while retaining the same vector. The rustyhood taxa are more or less localised, but when having the same pollinator it raises the question about their taxonomic levels. Some differ only in colour and appear to belong to the same taxon. Molecular work is likely to find only marginal differences between many others.

Populations of the widespread small-flowered *Pterostylis rufa* have similarly such many localised forms, that all have the same fungus-gnat *Orfelia* sp. 3 (Fig. 13) as their principal pollinator. They were found at low altitudes, near coastal habitats, up to moderate elevations in the foothills, ranging from the Brisbane ranges to East Gippsland.

Orfelia fungus-gnats were usually attracted to the orchids after 2:00 PM, in some areas until late in the afternoon during mild to warm conditions, and a few were active on very hot days. Many of the allopatric forms of undetermined rustyhood taxa have the same pollinator, suggesting that they are localised forms of the same taxon. The principal vectors have a similar length to the labellum of the rustyhood species they are attracted to, and as was to be expected, the smallest rustyhood *P. pusilla* attracted the smallest *Orfelia* member, sp. 4 (Fig. 14), matching the length of the labellum closely.

Discovering that rustyhood pollinators were usually active on sunny days and mainly in the afternoon, helped in detecting and observing interactions for additional species. An obstacle was the remoteness with populations in the west of the state. Of the taxa within a days traveling, making observations of the pollinator on *Pterostylis despectans* proved to be one of the most difficult, it took three seasons to capture images of the vector, and required camping overnight for success. Flowers were numerous at one site during the first season they were studied, but no fungus-gnats were attracted to the flowers and none were pollinated. The next season only one individual was seen flying around a flower, but



Fig. 11 Wing venation of *Orfelia*.



Fig. 12 *Pterostylis cheraphila* with *Orfelia* sp 1, its principal pollinator about 8 mm long.



Fig. 13 *Pterostylis rufa* with *Orfelia* sp 3, its principal pollinator 3.8 mm long.

it may have been the 'wrong' species. On the third season a seed-pod was found, but it took several more trips to finally witness a vector entering a flower and emerging with its pollinia (Fig. 15). Only a small number were observed very late in the day, as the sun went down and not surprisingly, it was another *Orfelia*, and recorded as sp. 5. Based on our observations, and the lack of setting fruit at the sites inspected, this fungus-gnat appears to be rare. Interestingly on two different visits an *Orfelia* sp. 1 was observed homing in and landing on flowers of *P. despectans*, but normally this species would be much too large to be a principal vector.

Orfelia sp. 2 (Fig. 16) pollinated both *Pterostylis boormanii* and *P. setifera*, and hybrids are known from several sites. Historically these two rustyhood species and their respective fungus-gnat vectors may both have been allopatric, but with most of their habitats greatly modified by gold diggings and clearing, the environment has changed completely. Habitat fragmentation and the historical effects of climate change can lead to further confusion in their geographical ranges.

In most locations pollinators appear to be absent as no seed-pods were found over many years, but with some species it took several seasons to observe activity. E.g. *Pterostylis hamata* is locally common near Beechworth, but no evidence of pollination was found, however due to the long travel distance only a few short visits were possible. Based on the length of the labellum it will most likely attract *Orfelia* sp 1 or a very close sibling.

Of the various taxa amongst the rustyhood-clade, flowers are basically the same in design, primarily varying in size, setae arrangement and colour, whilst labella lengths are in relation to the size of their vector. Differences between similar taxa are more evident in the plant habit, but if variability relates to environmental conditions or locality is not clear. Even the short-stemmed *P. despectans* has similar flowers to many members of the *Pterostylis valida*-group, that grow tall.

Pollinators typically home in on the kairomones emitted most strongly from the basal mound of the labellum. When set, the labellum is very sensitive and may trigger from small disturbances, even from the wind at times. It may slowly move to start with, then quickly accelerate, or it flicks up in one move. A pollinator positioned on it (e.g. Fig. 15) would become trapped, as the main entrance of the hood is blocked by the labellum. When with pollinia on the thorax, it may deposit pollen on the stigma, and collect a fresh pair, if present, via the column-wings escape route (see Fig. 9).



Fig. 14 *Pterostylis pusilla* with *Orfelia* sp 4. The smallest rustyhood with the smallest gnat-genus vector.



Fig. 15 *Pterostylis despectans* with *Orfelia* sp 5.



Fig. 16 *Pterostylis setifera* with the *Orfelia* sp 2. It also pollinates *P. boormanii*, causing hybrids.

Non-Sciarioidea pollinators

Two *Pterostylis*-clades did not attract fungus-gnats. 1/ *P. parviflora*-group, tiny greenhoods, that are multi-flowered and have food-related attractions. They have visitors comprising many different taxa over several families of micro-diptera.

2/ *P. plumosa*-group, the bearded greenhoods, that are single flowered with sex-related attractions. They are unique amongst the *Pterostylis* members in their vectors being daggerflies, family Empididae, attracting only males of *Empid* spp.

Tiny Greenhoods

Members of the Tiny Greenhood complex have particularly short lateral sepals and their attraction was found to be food-related. From watching vector behaviour, it is apparent that the scent is emitted from the lure inside the flower. The micro-dipteran pollinators observed belonged to several different families in the Langwarrin study-site alone. The tiny flies can be locally abundant, often pollinating new flowers on the same day, and pollinia are quickly taken. After landing on the flower, they usually walked into the opening as shown in Fig. 17, and showed no interest in the short lateral-sepals. Many flowers had pollinia removed by earlier visitors, but were not pollinated. The scent is emitted until setting fruit, and consequently vectors coming out of these flowers had no pollinia attached. In Langwarrin, in semi-shaded moist habitats, pollination normally occurs within a few days from the flowers opening. At a site near Ingleston, where there are some very large colonies growing in a dry gravelly habitat, no evidence of pollination was found, but visits were limited to once or twice during a season.

Of the tiny greenhoods monitored for pollinators, *Pterostylis parviflora* was observed regularly every season, and *P. aphylla* only occasionally. Vectors comprised tiny taxa, ranging from about 1 to 2.5 mm in body length, and of both sexes. The species were identified to family level and belonged to the Chloropidae, Phoridae and Scatopsidae. Many of the visitors attracted were often too large to enter a flower, and tried to feed with their long proboscis through the opening of the hood. A 2.5 mm long chloropid fly was the largest vector seen coming out of a *P. parviflora* flower with pollinia. The most efficient in collecting pollinia were members of Scatopsidae family, that ranged in body-lengths from about 1 to 2 mm. The smallest individual was photographed with a single pollinium on the thorax (Fig. 18), whilst the larger flies usually collected a pollinia pair (Fig. 19). The tiny-greenhoods seem to be the most primitive members of *Pterostylis* greenhoods in having multiple flowers on a stem and presumably a food-related attraction.



Fig. 17 *Pterostylis parviflora* with a Scatopsidae taxon about 2.0 mm long.



Fig. 18 *Pterostylis parviflora* with a Scatopsidae taxon about 1.0 mm long.



Fig. 19 *Pterostylis aphylla* and a Phoridae taxon about 1.8 mm long with fresh pollinia.

Bearded greenhoods

Of the *Pterostylis* clades, the bearded greenhoods are thought to be one of the most evolved. The two taxa recognised in Victoria are *Pterostylis plumosa* and *P. tasmanica*. They have a single flower and emit a sex-related scent to attract male pollinators. This clade evolved with free-moving labella, instead of being set to trigger and trap pollinators, as found in all the other *Pterostylis* members.

Pollinators were recently discovered on *P. plumosa* (Kuiter *et al*, 2017), which sexually attracted male *Empis* sp., of the dagger-fly family Empididae in which the males normally carry a nuptial gift to a female to be accepted for copulation. Every male observed presented it to the orchid flower (Fig 20) as well. Evidence of pollination had not been found for many seasons in the populations throughout the various regions checked and it seems that the *Empis*-fly pollinators have vanished in most places where the bearded greenhoods grow.

Fruit-setting in *Pterostylis tasmanica* is common, but was attributed to autogamy in the populations checked. Insect pollinators reported for this species needs to be confirmed. Populations in Langwarrin and Yarram were planned for pollinator studies, but before any observations could be made they were destroyed by prescribed burning. Other known populations are too remote to make regular visits, but efforts will be made to find out more about this taxon – if possible.

Summary

Of the diptera-pollinated orchids, species that attracted members of the Mycetophilidae family are thought to be the most specialised. The majority of these fly taxa were observed as principal or primary pollinators of the greenhood and rustyhood clades, attracting only males with kairomones. Members observed belonged to 6 genera: *Allodia*, *Leia*, *Mycetophila*, *Mycomya*, *Orfelia* and *Phthinia*. Single species of *Allodia* and *Phthinia* were found to be vectors on *P. diminuta* and *P. concinna* respectively. The latter greenhood has a secondary pollinator as well, a Sciaridae member, which is the probable cause of hybrids between closely related greenhoods, such as the well known Mentone Greenhood, named *P. Xtoveyana*.

The species considered to be the most primitive of the *Pterostylis* groups, are usually pollinated by both sexes of members in the Sciaridae family, known as dark-winged fungus-gnats. Ancestral forms of the greenhoods would have attracted their pollinators with a food-related scent and this has remained the case for the tiny-greenhood group. To a lesser degree it is evident in small single-flowered



Fig. 20 *Pterostylis plumosa* with a male *Empis* sp., presenting a nuptial gift.



Fig. 21 Wing venation of *Leia*.

Sexually attracted to the orchids, usually small taxa of the Mycetophilidae family, less than 3 mm long.

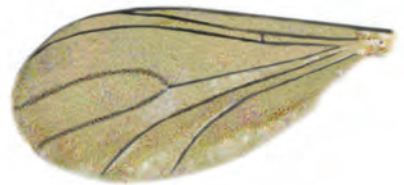


Fig. 22 Wing venation of Sciaridae.

Species, commonly known as dark-winged fungus-gnats, usually have dusky to near black wings. The various genera have very similar wing-venation and the figure shows the basic wing-design. Body colour is grey to black and the species observed on the orchids were about 3 mm long. Males are very slender and females have thick abdomen and ovipositor is usually visible.

greenhoods that attract both sexes of Sciaridae, such as *P. alveata* and *P. concinna*, but also have osmophoric sections on the lateral sepal tips that usually attract males of the Mycetophilidae family. Sciaridae vectors are often secondary pollinators with greenhoods that have a *Mycetophila* taxon as their primary vector, but only males were attracted to *P. cygnocephala* and *P. mutica* clade-members. These taxa lack lateral sepal tips and kairomones are presumably emitted from the distinct dark-green mound on the labellum.

About half of all terrestrial orchid species of Victoria rely on diptera vectors. The flies are very important to the health of environments, but do not get any consideration in the conservation efforts of orchids. All insects are suffering from habitat loss, and with environments being altered or ruined, the highly specialised species are the first to suffer. In many orchid habitats the pollinators have vanished from the regions. Modern agricultural techniques impact on invertebrates, while careless use of insecticides kills beneficial insects, including the pollinators and species that provide long-term pest control. The future looks bleak for orchids and insect pollinators alike, especially now many ecosystems in Victoria are being destroyed from frequently "prescribed burning". Fires can displace sensitive flora, but they are the most detrimental to orchids, in particularly the *Pterostylis* taxa. When fires are conducted too frequently, a rich habitat becomes depauperate and is converted to a fast growing fuel mass, increasing fire threats and reducing orchid habitat.

The primary pollinator of *Pterostylis concinna* is *Phthinia* sp 1, but apparently this species is now rare in many localities, and its Sciaridae secondary vector seems to have taken over. Several of the freshly pollinated flowers were checked and found to have a Sciaridae vector stuck on the stigma, with pollinia from another flower attached to the thorax. They were pollinated, but the vector had died in the process (Fig. 23). Female Sciaridae members are large enough to collect pollinia, but apparently not quite strong enough to pull free from the stigma when pollinating another flower. This observation suggests that the least specialised groups may be able to adopt different vectors when their primary pollinator vanishes from an area. The greenhood species that sexual attracted a *Mycetophila* male and also a Sciaridae member, but of both sexes,



Fig. 23 *Pterostylis concinna* with a dead female Sciaridae. It had entered with pollinia on thorax that hit the stigma and pollinated the flower, but was not strong enough to pull itself free. The principal pollinator is a male *Phthinia* sp 1, family Mycetophilidae.

seem to be in a transition stage, evolving from their food-related attractions to a more specialised species-specific strategy.

As shown in the lists of the fungus-gnat pollinators and *Pterostylis* taxa, there is a strong correlation between the fungus-gnat genera and the different *Pterostylis* clades. It also reflects the evolutionary developments in the most specialised groups being specific in vectors belonging to certain genera. The rustyhoods are a highly specialised group amongst the *Pterostylis* clades, and this is supported by their vectors belonging to the same fungus-gnat genus of the Mycetophilidae family. All were placed in the genus *Orfelia*, based on wing-venation. This genus has numerous members world-wide, but it may comprise many subgenera.

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Acknowledgements

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List of *Pterostylis* species and pollinators

1 Genus *Pterostylis* – Greenhoods

- 1 *P. acuminata*Mycetophilidae, *Mycomya* sp 2.
- 2 *P. Xaenigma*Mycetophilidae, *Mycomya* sp 2.
- 3 *P. alata*Mycetophilidae, *Mycetophila* sp 5.
- 4 *P. alpina*Mycetophilidae, *Mycomya* sp 5.
- 5 *P. alveata*Mycetophilidae, *Mycetophila* sp 2.
- 6 *P. alveata-peloric?*Sciaridae, Genus? sp 2.
- 7 *P. atrans*Mycetophilidae, *Leia* sp 2.
- 8 *P. baptistii*Mycetophilidae, *Leia* sp 3.
- 9 *P. coccina*Mycetophilidae, *Mycetophila* sp 6.
- 10 *P. concinna*Mycetophilidae, *Phthinia* sp 1. Sciaridae, Genus? sp 3.
- 11 *P. cucullata*Mycetophilidae, *Mycomya* sp 2.
- 12 *P. curta*Mycetophilidae, *Mycomya* sp 3.
- 13 *P. decurva*Sciaridae, *Trichosia* sp 1.
- 14 *P. dolichochila*Mycetophilidae, *Mycetophila* sp 5. Sciaridae, Genus? sp 4.
- 15 *P. falcata*Mycetophilidae, *Mycomya* sp 2.
- 16 *P. fischii*Mycetophilidae, *Leia* sp 1.
- 17 *P. grandiflora*Mycetophilidae, *Mycetophila* sp 3.
- 18 *P. grandiflora* var.Mycetophilidae, *Mycetophila* sp 8.
- 19 *P. Xingens*Mycetophilidae, *Mycomya* sp 2.
- 20 *P. lustra*Mycetophilidae, *Mycomya* sp 12.
- 21 *P. monticola*Mycetophilidae, *Mycomya* sp 4.
- 22 *P. nana*Sciaridae, Genus? sp 1.
- 23 *P. nutans*Mycetophilidae, *Mycomya* sp 2.
- 24 *P. obtusa*Mycetophilidae, *Mycetophila* sp 1.
- 25 *P. pedoglossa*Mycetophilidae, *Mycetophila* sp 4.
- 26 *P. pedunculata*Mycetophilidae, *Leia* sp 1.
- 27 *P. reflexa*Mycetophilidae, *Mycetophila* sp 9, Sciaridae, *Trichosia?*
- 28 *P. robusta*Sciaridae, Genus? sp 5.
- 29 *P. sanguinea*Mycetophilidae, *Mycomya* sp 6.

2 Genus *Pterostylis* – Tall Greenhoods

- 30 *P. diminuta*Mycetophilidae, *Allodia* sp 1.
- 31 *P. chlorogramma*Mycetophilidae, *Mycomya* sp 7.
- 32 *P. crassa*Mycetophilidae, *Mycomya* sp 11.
- 33 *P. melagramma*Mycetophilidae, *Mycomya* sp 9 & 10.
- 34 *P. smaragdina*Mycetophilidae, *Mycomya* sp 1.
- 35 *P. smaragdina* var. *prasina*Mycetophilidae, *Mycomya* sp 8.
- 36 *P. smaragdina* var. *prasina*, ChilternMycetophilidae, *Mycomya* sp 8.
- 37 *P. smaragdina* var. *rubrirostrata*Mycetophilidae, *Mycomya* sp 8.
- 38 *P. tunstallii*Mycetophilidae, *Mycetophila* sp 7.

3 Genus *Pterostylis* – Rustyhoods

- 39 *P. basaltica*Mycetophilidae, *Orfelia* sp 1.
- 40 *P. aff. biseta*Mycetophilidae, *Orfelia* sp 1.
- 41 *P. boormanii*Mycetophilidae, *Orfelia* sp 2.
- 42 *P. cheraphila*Mycetophilidae, *Orfelia* sp 1.
- 43 *P. despectans*Mycetophilidae, *Orfelia* sp 5.
- 44 *P. maxima*Mycetophilidae, *Orfelia* sp 1.
- 45 *P. pusilla*Mycetophilidae, *Orfelia* sp 4.
- 46 *P. rufa*Mycetophilidae, *Orfelia* sp 3.
- 47 *P. setifera*Mycetophilidae, *Orfelia* sp 2.
- 48 *P. aff. valida*Mycetophilidae, *Orfelia* sp 1.

4 Genus *Pterostylis* – Midget Greenhoods

- 49 *P. cynocephala*Sciaridae, Genus? sp 7.
- 50 *P. mutica*Sciaridae, Genus? sp 6.

5 Genus *Pterostylis* – Tiny Greenhoods

- 51 *P. aphylla*Chloropidae, Phoridae, Scatopsidae spp, various small flies.
- 52 *P. parviflora*Chloropidae, Phoridae, Scatopsidae spp, various small flies.

6 Genus *Pterostylis* – Bearded Greenhoods

- 53 *P. plumosa*Empididae, *Empis* sp 1.



List of fungus-gnat pollinators and *Pterostylis* species

Mycetophilidae,

1 Genus *Alodia*

- 1 *Alodia* sp 1*P. diminuta*

2 Genus *Leia*

- 2 *Leia* sp 1*P. pedunculata*
.....*P. fischii*
3 *Leia* sp 2*P. atrans*
4 *Leia* sp 3*P. baptistii*

3 Genus *Mycetophila*

- 5 *Mycetophila* sp 1*P. obtusa*
6 *Mycetophila* sp 2*P. alveata*
7 *Mycetophila* sp 3*P. grandiflora*
8 *Mycetophila* sp 4*P. pedoglossa*
9 *Mycetophila* sp 5*P. alata*
.....*P. dolichochila*
10 *Mycetophila* sp 6*P. coccina*
11 *Mycetophila* sp 7*P. tunstallii*
12 *Mycetophila* sp 8*P. grandiflora* var.
13 *Mycetophila* sp 9*P. reflexa*

4 Genus *Mycomya*

- 14 *Mycomya* sp 1*P. smaragdina*
15 *Mycomya* sp 2*P. acuminata*
.....*P. Xaenigma*
.....*P. cucullata*
.....*P. falcata*
.....*P. Xingens*
.....*P. nutans*
16 *Mycomya* sp 3*P. curta*
17 *Mycomya* sp 4*P. monticola*
18 *Mycomya* sp 5*P. alpina*
19 *Mycomya* sp 6*P. sanguinea*
20 *Mycomya* sp 7*P. chlorogramma*
21 *Mycomya* sp 8*P. smaragdina* var. *prasina*
.....*P. smaragdina* var. *prasina*, Chiltern
.....*P. smaragdina* var. *rubrirostrata*
22 *Mycomya* sp 9*P. melagramma*
23 *Mycomya* sp 10*P. melagramma*
24 *Mycomya* sp 11*P. crassa*
25 *Mycomya* sp 12*P. lustra*

5 Genus *Orfelia*

- 26 *Orfelia* sp 1*P. basaltica*
.....*P. aff. biseta*
.....*P. cheraphila*
.....*P. maxima*
.....*P. valida*
.....*P. aff. valida*
27 *Orfelia* sp 2*P. boormanii*
.....*P. setifera*
28 *Orfelia* sp 3*P. rufa*
29 *Orfelia* sp 4*P. pusilla*
30 *Orfelia* sp 5*P. despectans*

6 Genus *Phthinia*

- 31 *Phthinia* sp 1*P. concinna*

Sciaridae

- 32 sp 1*P. nana*
33 sp 2*P. alveata*-*peloric*?
34 sp 3*P. concinna*
35 sp 4*P. dolichochila*
36 sp 5*P. robusta*
37 sp 6*P. mutica*
38 sp 7*P. cyanocephala*

7 Genus *Trichosia*

- 39 sp 1*P. decurva*
40 sp ?*P. reflexa*

