Pollination of The Tuncurry Midge Orchid (Genoplesium littorale)

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INTRODUCTION

FloraSearch was commissioned by Landcom to investigate the pollination mechanism of the Tuncurry Midge Orchid, *Genoplesium littorale*, at North Tuncurry, NSW. A preliminary investigation was conducted in the field on one day (15 April) in 2012. Unfortunately, it was too late in the flowering period to conduct a full pollination study in 2012. Nevertheless, three inflorescences which had not completely finished flowering were collected for dissection of flower parts, and the percentage pollination of flowers on 18 finished inflorescences was determined. The results of the dissections and pollination measures were presented in a preliminary report (FloraSearch, 2012). The key findings were:

1. Two species of *Genoplesium* occur at North Tuncurry, the Tuncurry Midge Orchid, *G. littorale* and the Red Midge Orchid, *G. rufum*.
2. The proportion of flowers setting seed on 18 inflorescences varied from zero to 100 percent, averaging 34.6 percent. Similar levels of seed set occurred at the Tuncurry Tip and power line populations.
3. Microscope examination of flowers on two preserved inflorescences of *G. littorale* showed:
    a. They had high levels of pollinaria removal by insects, 77 (Tuncurry Tip) and 95 (power line) percent.
    b. The power line flowers had higher levels of pollination of the stigma (74%) and seed pod development (68%) than the Tuncurry Tip flowers (15 and 23 percent, respectively).
4. Observations of floral morphology, pollinaria removal and pollen deposition in *G. littorale* were all consistent with insect-mediated pollination.
5. The evidence did not support the existence of self-pollination (autogamy) or apomixy in *G. littorale*.

The investigations were continued in 2013 with the following aims:

1. To capture samples of *G. littorale* pollinators for identification.
2. To determine whether *G. littorale* has a single specific pollinator, a limited group of related pollinators, or a broad range of pollen vectors from many groups.
3. To determine whether the flowers of *G. littorale* emit an odour and/or produce nectar to attract pollinators.
4. To confirm by dissection of flowers that *G. littorale* is not self-pollinating (autogamous) or apomictic.
5. To assess the ecological requirements of the pollinators and estimate the minimum area needed to ensure the long term viability of pollinator populations.

This report presents the results obtained in 2013 and combines them with the 2012 results into a final report.

LITERATURE REVIEW

The Tuncurry Midge Orchid, *Genoplesium littorale*.

The Tuncurry Midge Orchid is a rare recently described species (Jones, 2001) that is listed as Critically Endangered under the NSW *Threatened Species Conservation Act* and the Commonwealth *Environment Protection and Biodiversity Conservation Act* (as *Corunastylis littoralis*).

G. littorale is a renascent terrestrial herb, with a single tubular leaf to 25 cm high from which emerges the single flower stem bearing from 5 to 30 small (5 × 4 mm) yellowish green flowers with dark reddish black extremities (Plate 1). A distinctive feature is the fleshy, purplish brown labellum with a prominent furrowed callus. All floral segments lack marginal hairs. Flowering takes place between

March and May, after which plants die back to the underground tuber. A new leaf emerges with good rains in late summer.

G. littorale appears to be confined to consolidated coastal sand dunes in a small area north of Tuncurry, NSW. The total population is estimated at less than 2000 plants with an area of occupancy less than eight km^2 (OEH, 2013).

Plate 1. Tuncurry Midge Orchid, *Genoplesium littorale*

Genoplesium Pollination

The following account is a slightly edited version of a review published by the author (Bower, 2001a).

Formal scientific studies on the pollination of *Genoplesium* are lacking. However, there have been a number of reports by naturalists that provide useful insights (Garnet 1940; Jones 1970; Bates 1981, 1988). The flowers of *Genoplesium* are small, inconspicuous and dull-coloured in shades of reddish brown, purple and green. The tepals and labellum may be fringed with cilia, the latter hanging loosely and waving freely in the breeze in some species. These characteristics conform most closely to myophily or fly pollination (van der Pijl and Dodson 1966). This is borne out by the limited data on pollinators which suggest *Genoplesium* is pollinated exclusively by small flies of the families Chloropidae and Milichiidae. Garnet (1940) indicates nectar is present in some *Genoplesium* species, indicating the pollination strategy involves nectar reward. A few species are autogamous, that is, self-pollinating (Jones 1972; 1998) and one is apomictic, that is, development of seed occurs without fertilisation (Jones 1977; Jones and Clements 1989)

Jones (1972) reported that *Genoplesium nudum* (as *Prasophyllum beaugleholei*) and *G. pumilum* (as *P. aureoviride*) are autogamous. He also noted (Jones 1998) that populations of *G. archeri* in south west Tasmania are autogamous whereas the species is entomogamous through the rest of its large range. *G. nudum* has a suite of characteristics typical of autogamous orchids (Jones 1972). The flowers are short-lived, lasting only two to three days after anthesis. The pollinia lack coherence,

even in the bud, and are only weakly attached to the viscidium, which lacks a viscid secretion and is unlikely to be removed by an insect. All ovaries on all plants swell and contain viable seed by contrast to outcrossing species in which many ovaries are not fertilised.

The mechanism of self-pollination in *G. nudum* is simple, but does not commence until the flower has opened (Jones 1972). The anther is located behind the narrow upper half of the stigma and is separated from it by the rostellum in the early bud. Two days before the flower opens the rostellum moves forward of the anther, the pollinia are incoherent and the stigma has become moist but not sticky. After the flower opens the anther sacs split wide open, the pollinia rest on the back of the rostellum which has bent further forward in front of the stigma to an angle of 45 degrees or less, and pollen grains begin to fall on the now sticky stigmatic surface. As flowering progresses pollination appears to involve two processes; the loose pollen grains bubble over the rostellum onto the stigma and the stigma grows around the rostellum to meet them. The stigma ultimately becomes very swollen and distorted.

Apomixy has been proposed for *Genoplesium apostasioides* (as *Prasophyllum anomalum* and *P. bowdeniae*) (Jones 1977; Jones and Clements 1989) which includes a wide variety of abnormal forms with deformed flowers and abortive columns. Swelling of the ovaries begins in the bud stage and is well advanced by anthesis which is short or foregone. The stigma and anther may be on different processes and the anther may lack pollen altogether, or if it is present, is tightly bound and cannot be removed.

The dominant pollination mechanism in *Genoplesium* appears to be xenogamy or geitonogamy mediated by small flies. The early observations of Garnet (1940) remain the most thorough and complete pollination study of the group so far. Garnet (1940) studied four species near Melbourne in Victoria, though their exact identity is uncertain due to recent taxonomic revisions (Jones and Clements 1989; Jones 1991; Jones and Jeanes 1996; Jones 1998). Over several seasons Garnet (1940) observed the behaviour of flies visiting *G. morrisii*, *G. archeri* (but possibly *G. ciliatum*), *G. nigricans* (probably an undescribed species related to *G. rufum* (Backhouse and Jeanes 1995) and *G. despectans*. Other observations are those of Jones (1970) on five species, and Bates on *G. ciliatum* (as *Prasophyllum archeri*) (Bates 1981) and *G. acuminatum* (Bates 1988).

The attraction of flies to some *Genoplesium* species is very strong and it is common for several to many flies to swarm over fresh inflorescences (Garnet 1940; Bates 1981, 1988). Flies respond rapidly to bait flowers placed in the field; Bates (1981) noted a response by seven flies within one minute of a pot of *G. ciliatum* flowers being placed out. Such rapid responses are similar to those of pollinators sexually attracted by pseudo sex pheromones (Peakall 1990). Attraction to *Genoplesium* appears to be by odours, not all of which may be detectable by humans. Garnet (1940) could only detect an odour in *G. despectans*, but not *G. morrisii*, *G. archeri*, or *G*. aff. *rufum*. *G. fimbriatum* has a strong lemon scent which increased in intensity with rising temperature (Jones 1970). Blaxell (1970) reported *G. apostasioides* (as *Prasophyllum anomalum*) has a faint lemon scent, *G. archeri* smells of sour milk, *G. citriodorum* (as *P. morrisii*) has a very strong lemon fragrance (see also Jones 1991) and *G. simulans* (as *G. morrisii* var. *intermedium*) has a weak lemon scent mixed with an ant-like aroma, though Jones (1991) indicates *G. simulans* lacks a lemon fragrance. Blaxell (1970) detected no odour in *G. nudiscapum* (as *P. densum*), *G. pumilum* (as *P. aureoviride*) and *G. nudum* (as *P. beaugleholei*); the latter two species are autogamous so the lack of an odour is not surprising. Although flowers of *G. acuminatum* were actively visited by flies no odour could be detected (Bates 1988).

The available records of visitors to *Genoplesium* species all involve flies of the closely related families Chloropidae and Milichiidae suggesting *Genoplesium* is specifically adapted to these fly families as pollinators. Specimens collected by Garnet (1940) were identified as belonging to four or five species in three genera and two families, but only three were named, all chloropids, as follows: *Caviceps flavipes*, *Oscinosoma subpilosa* and an undescribed species of *Oscinsoma*. The specific orchid species visited by each fly species were not given. A photograph in Cady and Rotherham (1970)

shows a chloropid bearing pollinia on the labellum of *G. archeri* (as *Prasophyllum archeri*) and captioned as *Conioscinella becker*. The reliability of the identification cannot be assessed since no details of the observation are given in the text. The identities of the insects observed by Jones (1970) and Bates (1981, 1988) were not given, but the flies collected by D. L. Jones were subsequently identified by D. Colless (unpublished) as follows: species of *Caviceps* on *G. nigricans*, *G. despectans*, *G. morrisii* and *G. rufum*; *Caviceps flavipes* was also collected on *G. rufum*. As an additional unpublished record, flies caught by A. E. Logan on *G*. aff. *rufum* at Carabost, New South Wales, were identified by D. K. McAlpine of the Australian Museum as chloropids of the genus *Lioscinella* and milichiids of the genus *Stomosis*.

The mechanism of insect mediated pollination in *Genoplesium* was described in detail by Garnet (1940). Attracted flies landed on the inflorescence and moved to the downward hanging labellum which they gradually walked up, probing with their probosces as they went. Garnet (1940) noticed the prominent raised callus plate of the labellum exuded droplets of nectar which the flies seemed to imbibe. Once on the labellum the flies became totally absorbed and were unperturbed by close observation with a hand lens or even inversion of the flowers (Garnet 1940; Bates 1981). The flies moved to the base of the labellum (Garnet 1940) forcing their way below the rostellum by jerking movements of the legs (Bates 1981, 1988) where they spent up to several minutes. In this position the fly's thorax contacted the viscidium. After flies have finished on one flower they may move to others on the same raceme (Garnet 1940) suggesting geitonogamous self-pollination occurs. This behaviour also suggests the flies are deriving a reward for their efforts (Bates 1988).

The available data do not allow definite conclusions to be made about the degree of pollinator specificity in *Genoplesium*. Garnet (1940) did not report which species of flies were attracted to each *Genoplesium* species, but considered pollinators were shared among species allowing the possibility of hybridisation. However, hybrids were not apparent in mixed populations of *Genoplesium* species she examined. By contrast, observations by Jones (1970) and Bates (1988) suggest some level of specificity may occur. Jones (1970) observed that small flies behaved differently towards five species of potted *Genoplesium* placed together in a backyard. The flies removed the pollinaria of only one species, *G. morrisii*, but also actively worked the flowers of *G. despectans*. They landed on the inflorescence of *G. fimbriatum*, but did not enter the flowers, and showed little interest in *G. nigricans* (as *Prasophyllum fusco-viride*). The flies ignored *G. filiforme* (as *P. nublingii*) altogether. Similarly, Bates (1981) observed that larger flies visited *G. ciliatum* than went to *G. nigricans* and *G*. aff. *rufum* in the same glasshouse over the same time period. However, Bates (1988) also found that the same unidentified fly species visited *G. acuminatum* and *G. ciliatum* in the same glasshouse. It should be noted that *G. acuminatum* and *G. ciliatum* do not occur sympatrically, the former is found in coastal northern New South Wales and Queensland, and the latter in southern Victoria and South Australia. It appears that allopatric *Genoplesium* taxa, which have no opportunity to hybridise, may attract the same pollinators.

Hybrids have been reported among some *Genoplesium* species indicating that pollinator specificity is incomplete. Hybrids between *G. ciliatum* (as *Prasophyllum archeri*) and *G. despectans* (as *P. despectans*) have been reported by Bates and Weber (1979), while Backhouse and Jeanes (1995) report that *G. archeri s. s.* also hybridises with *G. despectans*. Jones (1991) indicates that hybrids may occur between *G. citriodorum* and *G. simulans*, two closely related species in the *G. morrisii* complex, but only in disturbed sites.

METHODS

Location of study area and subject plants

The study was undertaken at the main known population sites of *G. littorale*; Chapmans Road (Figure 1) and Tuncurry Waste Management Centre (Figure 2). The population near Chapmans Road occurs sporadically along the edge of a mown power line easement. The population east of Tuncurry Tip occupies parts of a rehabilitated sand mining path and is much larger than at Chapmans Road. A total of 141 plants were individually tagged with small plastic horticultural pot tags placed approximately 10 cm from the plant with the label facing it. The following information was recorded for each plant:

- The presence of closed (finished) flowers, open flowers and buds at the time of tagging (12 and 13 March 2013).
- The identity of pollinators captured.
- The numbers of seed pods and unpollinated flowers at the end of the flowering season (23 April 2013).

Plants were tagged in four groups, mainly on March 12 and 13, 2013, as shown on Figures 1 and 2, and Table 1.

Table 1.
Grouping of Tagged Plants for Pollinator Observations and Seed Pod Assessment.

Group	Location	No. of plants
A	Chapmans Road	34
B	South side of North Boundary Fire Trail (east of Tuncurry Tip)	20
C	North side of North Boundary Fire Trail (east of Tuncurry Tip)	57
D	South of track to south end of Darawank Nature Reserve (east of Tuncurry Tip)	30
Total		141

At the time of marking 73 percent of plants had open flowers and 27 percent were still in full bud with no open flowers. Pollination had commenced with developing seed pods present on 9 percent of plants and 30 percent having closed flowers that had likely been pollinated. Unopened buds were present on 51 percent of plants with open flowers.

Pollinator observations and capture

The flowers of individual marked plants in each sub-population were examined closely for the presence of pollinators for approximately 10 to 15 seconds per plant. Each plant was visited three or more times daily on March 12, 13 and 14, 2013 when temperatures were higher than 20 degrees centigrade. The following information was recorded for each pollinator observed or captured:

- The tag number of the plant on which it was observed.
- The time of the observation.
- Whether it was cloudy or sunny.
- The air temperature (using a digital thermometer; [Kestrel 3000 Pocket Weather Meter])
- Whether or not the insect was carrying orchid pollinaria on its body.

Insects were captured with an aspirator, which involves sucking them through a plastic tube into a glass vial. Captured insects were transferred from the vial into smaller tubes containing 70% ethanol for preservation. The tubes were labelled in the field with the date and *G. littorale* plant number.

Captured insects were taken to Dr. Dan Bickel of the Australian Museum in Sydney for identification.

Figure 1. Locations of marked *Genoplesium* plants in Group A, Chapmans Road, Tuncurry, NSW.

Figure 2. Locations of marked *Genoplesium* plants in Groups B, C and D, east of Tuncurry Waste Management Centre, NSW.

Collection and examination of inflorescences

Six whole inflorescences of *G. littorale* with closed flowers, open flowers and buds were preserved in 70 percent ethanol for later detailed microscope examination. The inflorescences were examined with a binocular dissecting microscope at magnifications up to 40 times. The following information was recorded for each flower:

- Whether the pollinarium (viscidium plus pollinia) was present in the anther sacs or had been removed by a pollen vector.
- Whether any pollen had been placed on the stigma, and if so, whether it was a small, medium or large amount.
- Whether the ovary was swollen.
- If the pollinarium remained in situ, whether there was any evidence of self-pollination, such as growth of pollen tubes into the stigma from the anthers, or the spilling of pollen from the anthers onto the stigma, or outgrowth of the stigma to contact the pollinia.

Determination of *Genoplesium* species present

Observations by RPS (unpublished) and the author in 2012 indicated that the *Genoplesium* population on the study area comprises two species, *G. littorale* and a similar species thought to be the Red Midge Orchid, *G. rufum*. The relative proportions of the two on the study area are unknown. Accordingly, the level of the Red Midge Orchid in the population was estimated. This has importance for understanding whether or not particular pollinators are specific to one or the other orchid species. Plants were examined during tagging and, as a more rigorous check, single flowers were taken from 29 marked plants and five plants from a different area for microscope examination. The flowers were preserved in 70% ethanol in individual marked vials.

G. rufum has been identified for the study area on the basis of globular white glands on the tips of the lateral sepals, which are obvious in the plant shown in Plate 2. The identification of *G. rufum* was confirmed by Dr Peter Weston of the Royal Botanic Gardens and Domain Trust for specimens submitted by RPS (Isaac Mamott, pers. comm.). However, *G. rufum* is part of a complex of similar taxa, including *G. littorale* (Jones, 2006), whose members may be difficult to identify.

The diagnostic features listed in Table 2 are taken from Jones (2006) treatment of *Genoplesium*. Table 2 reveals only a few characters that may potentially distinguish these species. Flower colour appears to be one of these, but the stated colours do not agree well with the photographs used to illustrate the species in Jones (2006). Another inconsistency is that glands on the tips of the lateral sepals are not given as a diagnostic feature of *G. rufum* by Jones (2006), although he does mention in the description that 'tiny vestigial glands' may be present. This appears inconsistent with some plants at Tuncurry which may have prominent glands (Plate 2). Also confusing is no mention of lateral sepal glands in the description of Blackish Midge Orchid, *G. trifidum*, in Jones (2006), even though they are clearly visible in the photograph of the species. The role and importance of lateral sepal glands in this group seems unclear. The labellum is the only remaining organ that appears to have diagnostic potential. *G. littorale* has a purple-brown labellum with irregular margins; in *G. rufum* it is whitish or pinkish with a black callus and irregular margins, while *G. trifidum* has minutely toothed margins (Jones, 2006). Owing to the above inconsistencies the Tuncurry *G. rufum s.l.* plants are referred to hereafter as *G. rufum sensu lato*, abbreviated to *G. rufum s.l.*, where *sensu lato* means 'in the broad sense'.

A potentially useful character for *G. littorale* observed in one plant in 2012 (FloraSearch, 2012) is that the base of the shallow longitudinal groove in the labellum callus is shiny and has elongated epidermal cells compared with the callus ridges. This feature was absent from the deeper groove in a plant of *G. rufum s.l*.

Table 2
Diagnostic characteristics of species in the *Genoplesium rufum* complex that occur on the central and lower north coast of NSW (after Jones, 2006)
[Black text are diagnostic features, red text is from the description. Blue text is from the original taxonomic description of *Genoplesium littorale* (Jones (2001)]

Character	*G. littorale*	*G. rufum*	*G. trifidum*
Habit	Slender; flowers semi-nodding, green; coastal vegetation	Slender; flowers nodding, pinkish or reddish	Robust; flowers semi-nodding, dark purplish
Lateral sepal	Divergent, deflexed, base humped	Divergent, sharply pointed, or with tiny vestigial gland, base humped	Divergent, sharply pointed, base humped
Dorsal sepal	Apex sharply pointed	Sometimes with dark bands	With dark bands, apex drawn out
Petal	Spreading, sharply pointed	Apex drawn out, sharply pointed	Often with a whitish gland or pointed
Labellum	Oblong with sharply pointed recurved apex, purple-brown, margins irregular, fleshy	Obovate with slightly irregular margins, fleshy, whitish or pinkish with black callus, apex sharply pointed, recurved	Ovate, fleshy, with minutely toothed margins, apex recurved and sharply pointed, fleshy

Plate 2. *Genoplesium rufum s.l.* ex Tuncurry

Seed set data

All labelled plants were scored in the field for the presence of developing seed pods on 23 April 2013. Each finished flower was examined sequentially from the bottom of the inflorescence to the top and scored as to whether or not the ovary was swollen, indicating seed pod development. Seed pods can be distinguished from closed unpollinated flowers by the swelling of the ovary which projects outwards with the withered flower held away from the stem (Plate 3). By contrast, withered unpollinated flowers hang downwards against the stem (Plate 3).

Plate 3. *Genoplesium rufum s.l.* showing contrast between the swollen ovaries of developing seed pods and unpollinated flowers.

RESULTS

***Genoplesium* species present**

Flowers from 41 plants were examined microscopically, and an additional 9 inflorescences were macro-photographed. In addition to 34 randomly selected single flowers, flowers on the six whole *G. littorale* inflorescences were examined, as well as on one inflorescence identified in the field as *G. rufum s.l.* Unfortunately, preservation of flowers in alcohol resulted in complete leaching of colour so that colour could not be assessed. Colour is potentially an important labellum character.

Flowers in six samples and one photograph bore glands at the tips of the lateral sepals, are putatively *G. rufum s.l.* and represent 12.5 percent of the total population in the study area (excluding five outside plants of *G. littorale* and the non-randomly selected *G. rufum s.l.* from the calculation). *G. rufum s.l.* occurred in all four groups, spanning the whole distribution of *Genoplesium* on the study area. Only one plant with sepal glands, which were particularly prominent as per Plate 2, was observed in the field. Other plants had smaller less conspicuous glands (Plates 3 and 4), possibly equivalent to the 'tiny vestigial glands' referred to in *G. rufum* by Jones (2006). However, the plant in

Plate 4 bears a strong resemblance to the photo of *G. trifidum* in Jones (2006), which shows similar small sepal glands.

Plate 4. *Genoplesium rufum s.l.* with vestigial sepal glands, Group A.

Potential distinguishing features of the labellum were either difficult to discern or showed overlapping variation between plants with and without sepal glands. For example, the integrity of the labellum margin varied from entire to erose to a few having distinct short teeth. Of the six plants with sepal glands, five had slightly erose labellum margins and one was erose. The remaining plants, comprising *G. littorale*, had labellum margins that varied between entire (albeit irregular), erose, and shortly toothed, by contrast with Jones (2001) who described the margin as irregular. Jones (2006) also described the labellum margin of *G. rufum s.l.* as irregular, but plants with sepal glands in this study mainly had slightly erose margins, which may equate to his irregular margins. It is interesting that Jones (2006) describes *G. trifidum* as having minutely toothed margins. Plants with sepal glands on the study area seem to lie somewhere between *G. trifidum* and *G. rufum s.l.* in that their margins are irregular and slightly erose, but lack distinct teeth

The most useful labellum character found in this study is the differentiation of epidermal cells along the labellum groove (Plate 5). The groove cells are smooth, shiny and elongated in *G. littorale* and undifferentiated in *G. rufum s.l*. However, this character can be hard to distinguish in unstained preserved specimens, although it is obvious in living material (Plate 5). In four plants with sepal glands there was no evidence of cell differentiation, but it appeared to be present in the other two, suggesting possible hybridisation. Conversely, in some *G. littorale* plants, i.e. lacking sepal glands, cell differentiation was hard to see, although obvious in most.

Despite the difficulty in finding reliable characters in preserved specimens that correlate with the presence or absence of sepal glands, *G. littorale* presents as a clearly distinctive entity in photographs (Jones, 2001, 2006; Paget, 2008; OEH, 2013 and herein). It has more compact flowers than *G. rufum s.l.* with contrasting mostly green dorsal sepal and dark maroon to blackish lateral sepals. Its lateral sepals are also moderately divergent, short, mostly straight and lack glands, rather than being widely divergent, long and bowed with glands (Plate 1 versus Plates 4 and 5). The purplish labellum with shiny groove is most distinctive (Plate 5). These characters are best used for identification in living material.

Plate 5. Shiny central groove in the labellum of the Tuncurry Midge Orchid (upper flower).

Flower dissections

Three inflorescences were collected in 2012 and 7 in 2013 for microscopic examination of the pollination mechanism. Of these, 7 were *G. littorale* and 3 were *G. rufum s.l.* (Table 3). All flowers and mature buds on each inflorescence were examined for the presence or absence of pollinaria, the presence of pollen on the stigma, and whether a seed pod had developed. The results are summarised in Table 3.

The examinations showed that whole pollinaria were removed completely from the anthers of flowers of both species, consistent with removal by an insect pollen vector. No cases of partial removal of pollinia were observed, nor was there any evidence of pollinia breakdown in the anthers as might occur if the flowers were self-pollinating. Quite high levels of pollinaria removal had occurred in some plants, up to 90 percent (range 28% to 90% in *G. littorale* and zero to 44% in *G. rufum s.l.*) (Table 3). Pollen was commonly found abundantly on the stigma of flowers (15% to 79% in *G. littorale* and zero to 40% in *G. rufum s.l.*), many of which had swollen ovaries (15% to 68% in *G. littorale* and zero to 6% in *G. rufum s.l.*). No cases of pollen spillage from anthers onto the stigma were found, or growth of pollen tubes through the back of the stigma, or other mechanisms of self-pollination. In addition, swelling of ovaries only occurred where stigmas had been pollinated, ruling out apomixy. The observations are consistent with insect mediated pollination in both species.

Table 3
Pollination status of dissected flowers

Species [Plant no. (year)]	Flower status (number)				Pollinaria removal		Pollen on stigma		Swollen ovaries	
	Open	Closed	Buds	Total	No.	%	No.	%	No.	%
G. littorale										
1 (2012)	4	15	1	20	18	90.0	14	70.0	13	65.0
2 (2012)	0	13	0	13	10	76.9	2	15.4	3	23.1
3 (2013)	10	2	1	13	5	38.5	3	23.1	2	15.4
4 (2013)	5	10	0	15	6	40.0	4	26.7	4	26.7
5 (2013)	7	17	1	25	22	88.0	17	68.0	17	68.0
6 (2013)	9	6	3	18	5	27.8	6	33.3	5	27.8
7 (2013)	5	8	1	14	8	57.1	11	78.6	6	42.9
G. rufum s.l.										
1 (2012)	3	0	3	6	0	0.0	0	0.0	0	0.0
2 (2013)	5	10	1	16	7	43.8	3	18.8	1	6.3
3 (2013)	2	2	1	5	1	20.0	2	40.0	0	0.0

Odour and nectar

No odour was detected when *G. littorale* inflorescences were smelt in warm conditions (25 to 30 degrees C). Nor was nectar observable on the labellums of fresh flowers in inflorescences that were picked for microscope examination. Open flowers were photographed at 1 to 1 magnification on five inflorescences. No nectar was detected on the labellums of these flowers when digitally magnified on a computer screen (Plates 5 and 6). It appears that *G. littorale* does not produce large quantities of nectar, or any at all, suggesting it is potentially a food deceptive species, i.e. it attracts insects seeking food, but offers no food reward for the pollination service. This contrasts with the *G. rufum s.l.*, which provides copious nectar in its labellum groove (Plate 7).

Plate 6. Labellum of Tuncurry Midge Orchid showing lack of nectar droplets in the groove of the labellum callus.

Plate 7. Labellum of *G. rufum s.l.* showing a line of nectar droplets in the groove of the labellum callus.

Pollinators

Weather conditions were ideal for pollinator activity between 12 and 14 March. Maximum temperatures on the study area reached 28.9, 28.1 and 30.0 degrees centigrade on 12, 13 and 14 March, respectively, which is ideal for insect activity. Fifty one potential pollinators, all tiny flies, were observed on *Genoplesium* inflorescences. Nineteen (38%) of these were carrying pollinaria on the dorsal thorax. Twenty two were captured and identified, including ten with pollinaria.

The captured flies belonged to five species in the family Chloropidae. They keyed to two genera, *Cadrema* (1 species) and *Conioscinella* (4 species) (Table 3) using Wheeler (2010). The Australian Chloropids are a neglected group with many undescribed species, so it was not possible to identify the specimens beyond the generic level and even the generic placement of some *Conioscinella* specimens is uncertain (D. Bickel, pers. comm.). Table 3 gives the number of specimens and distinguishing characteristics of the five species.

Table 3.
Characteristics of five Chloropid fly species
attracted to *Genoplesium* species at Tuncurry, NSW

Chloropid species	No. of specimens	Diagnostic features
Cadrema sp. 1	2	Tibia III with long curved apical spine; subrectangular antenna
Conioscinella sp. 1	11	Distal frons and gena yellow; tibia II & III with banded appearance; antenna yellowish
Conioscinella sp. 2	2	Distal frons yellow; antenna dark brown
Conioscinella sp. 3	6	Distal frons black; antenna rounded, yellow; very small, < 1.0 mm
Conioscinella sp. 4	1	Distal frons black; antenna black

Three Chloropid species, *Cadrema* sp. 1, *Conioscinella* sp. 1 and *Conioscinella* sp. 3, carried *Genoplesium* pollinaria on the thorax (Table 4), thereby confirming them as *Genoplesium* pollinators. Eight pollinators carried a single pollinarium, but two had two pollinaria (Table 4). Most of the flies captured were females; 17 females to 5 males. The bias in favour of females was even greater among flies with pollinaria; 9 females to 1 male. These data suggest that females are more attracted to *Genoplesium* than males. Nevertheless, the fact that males were captured indicates that both sexes are attracted, precluding the possibility that attraction is associated with sexual deception, which always involves the deceit of males.

Table 4.
Chloropid visitors to *Genoplesium*:
Orchid species, presence of pollinaria and area of capture

Chloropid species	Sex	Confirmed *Genoplesium* species	No. with Pollinaria	Area	Comment
Cadrema sp. 1	2♀	*G. littorale*	2	A, C	
Conioscinella sp. 1	8♀	*G. littorale*	5	A, B, C, D	One specimen with 2 pollinaria
	3♂	*G. littorale*, *G. rufum s.l.*	-	B, D	
Conioscinella sp. 2	1♀	*G. littorale*	-	B	
	1♂	*G. littorale*	-	B	
Conioscinella sp. 3	5♀	*G. littorale*	2	B, C, D	One specimen with 2 pollinaria
	1♂	-	1	D	
Conioscinella sp. 4	1♀	-	-	A	

The two most common Chloropids in the collection, *Conioscinella* sp. 1 and *Conioscinella* sp. 3, were also the species with most pollinaria (Table 4), suggesting they are the dominant pollinators of *G. littorale* on the study area. However, although less common, both *Cadrema* sp. 1 specimens bore pollinaria, indicating this species is an effective pollinator. While *Conioscinella* sp. 2 and *Conioscinella* sp. 4 were uncommon and the specimens lacked pollinaria, they are potential pollinators, since they are attracted to *G. littorale* and are similar in size to the confirmed pollinator species.

Genoplesium species attracting pollinators

The unexpectedly high proportion of *G. rufum s.l.*, 12.5 percent, in the *Genoplesium* population on the study area has complicated interpretation of the pollinator results. Fortunately, 13 of the pollinators were captured on plants confirmed as either *G. littorale* or *G. rufum s.l.* by specimens or photographs (Table 4). All three species bearing pollinaria were captured on confirmed *G. littorale* plants and one of these, *Conioscinella* sp. 1, was also captured on a confirmed *G. rufum s.l.* plant.

Pollinarium position

Pollen in orchids is aggregated into masses called pollinia. The pollinarium in *Genoplesium* is a four part structure comprising two pollinia connected to a viscidium by a narrow stipe (Figure 3). The viscidium is a sticky disc-like structure that attaches to the pollinator when it enters the flower and presses against it. The withdrawing insect with attached viscidium pulls the pollinia from the anther sacs.

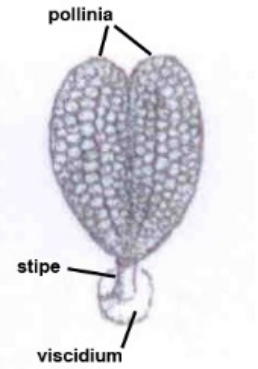

Figure 3. Structure of the *Genoplesium* pollinarium.

In *Genoplesium* the viscidium attaches to the centre of the thorax which contacts it as the fly straddles the labellum groove seeking nectar. The precise position of the viscidium on the thorax depends on the size of the fly and whether it has already picked up a pollinarium from another flower. In seven flies with single pollinaria, the viscidium was centred on the bilateral centre line of the dorsal thorax (Plate 8), while in another it was placed to the left of the centre line. Flies with two pollinaria had one straddling the centre line and the others displaced to the right side; one immediately to the right, and the other forward and to the right.

Plate 8. *Conioscinella* sp. 3 with *Genoplesium* pollinarium

On five flies the viscidium was attached in the centre of the mesonotum (thorax). However, in two others it was attached towards the rear of the mesonotum and in three it extended onto the scutellum, a projection of the thorax that extends over the front of the abdomen.

Pollinators may spend a considerable amount of time on the outside of flowers attempting to remove the pollinarium from their thorax by pushing backwards against the stipe or pollinia with their raised hind legs (Plates 9 and 10).

Plate 8. *Conioscinella* sp 3 attempting to remove Tuncurry Midge Orchid pollinarium

Plate 9. Undetermined pollinator attempting to remove Tuncurry Midge Orchid pollinarium

Weather conditions

Pollinators were observed and captured between 8.30 am and 4.20 pm Eastern Daylight Saving Time. Temperatures varied between 19.8 and 30.0 degrees C with most activity above 25 degrees C. Pollinators were active in both sunny and light to medium cloudy conditions.

Pollination success

Plant survival

The pollination success of tagged *Genoplesium* plants was determined on 23 April 2013. The raw field data for each plant are given in Appendix A. Of the original 141 plants, seven were sampled for dissection and three tags were not relocated, leaving 131 plants for assessment (Table 5). Of these, only 60, or less than half of the sample (45.8%) remained in a viable condition on 23 April (Table 5). A quarter of the plants (24.4%) were lost to herbivory, probably by macropods which nipped off the inflorescences and varying proportions of the stem. Another quarter (25.2%) of the plants was missing altogether, i.e. no above ground parts remained. It is likely that most of these were also lost to herbivory, suggesting that up to half the plants were eaten before they could produce seed. A small proportion of plants (3.8%) had shrivelled inflorescences for unknown reasons.

Table 5
Fate of marked *Genoplesium* plants

Group	Sampled	Herbivory		Missing		Shrivelled		Extant		Total[1]
		No.	%	No.	%	No.	%	No.	%	
A	2	4	12.5	6	18.8	1	3.1	21	65.6	32
B	1	10	52.6	6	31.6	1	5.3	2	10.5	19
C	3	13	25.5	18	35.3	2	3.9	18	35.3	51[2]
D	1	5	17.2	3	10.3	1	3.4	19	65.5	29
Total	7	32	24.4	33	25.2	5	3.8	60	45.8	131

[1] Excluding sampled plants; [2] Excludes three plants that were not relocated on 23 April.

Levels of herbivory were much higher in groups B and C than in groups A and D (Table 5). Group A is close to a main road and a residential area, which may discourage herbivores, especially macropods. Group D is characterised by a very open and sparse understorey with little cover for macropods and low levels of forage. By contrast, Groups B and C have relatively dense shrub cover for macropods and are remote from roads and residential areas.

Seed set

The proportions of flowers setting seed pods on the surviving 60 plants are given in Appendix A, Table 6 and Figure 4, and varied widely from zero to 100 percent (Appendix A, Figure 4), with an overall average of 42.6 percent across the whole study area (Table 6). Seed pod development was highest in groups B and D at 58.1 and 58.7 percent, respectively, although there were only 2 extant plants on area B (Table 6). Percentage seed pod set in groups A and C was almost half that in groups B and D, suggesting these areas may have had lower pollinator populations.

The data in Table 6 are amalgamated for all marked plants on the study area, comprising approximately 87.5 percent *G. littorale* and 12.5 percent *G. rufum s.l.*. The data from plants confirmed as *G. littorale* or *G. rufum s.l.* by microscope examination of single flowers is presented in Table 7. The mean percentage of flowers developing seed pods is similar for the two species;

42.3 percent for *G. littorale* (n=14) and 40.9 percent for *G. rufum s.l.* (n=3), which are comparable to the overall pollination level of 42.6 percent for all monitored plants across the study area. This result suggests there is no difference in the attractiveness to pollinators between *G. littorale* and *G. rufum s.l.*. It also suggests they may be utilising the same pool of insects and that there is potential for hybridisation.

Table 6
Seed set in *Genoplesium*, North Tuncurry, NSW

Group	No. of extant plants	Total viable flowers	Mean flowers / plant	Seed pods			Unpollinated flowers		
				Total	Mean / plant	Percent overall	Total	Mean	Percent overall
A	21	215	10.2	65	3.1	30.2	150	7.1	69.8
B	2	43	21.5	25	12.5	58.1	18	9.0	41.9
C	18	212	11.8	74	4.1	34.9	138	7.7	65.1
D	19	225	11.8	132	6.9	58.7	93	4.9	41.3
Total	60	695	11.6	296	4.9	42.6	399	6.7	57.4

Table 7
Percent seed pod development in individual plants confirmed as *G. littorale* or *G. rufum s.l.*

Group	Plant No.	Total viable flowers	Seed pods		Unpollinated flowers	
			No.	%	No.	%
G. littorale						
A	4	2	0	0	2	100
A	27	9	3	33.3	6	66.7
A	29	7	1	14.3	6	85.7
A	32	14	3	21.4	11	78.6
A	33	11	7	63.6	4	36.4
B	20	22	15	68.2	7	9.1
C	31	8	5	62.5	3	37.5
C	51	8	0	0	8	100
C	55	10	5	50	5	50
D	3	16	6	37.5	10	62.5
D	4	12	7	58.3	5	41.7
D	7	9	4	44.4	5	55.6
D	17	13	11	84.6	2	15.4
D	27	11	6	54.5	5	45.5
Total		152	73	592.6	79	784.7
Mean		10.9	5.2	42.3	5.6	56.1
G. rufum s.l.						
A	30	12	6	50	6	50
B	17	21	10	47.6	11	52.4
D	22	12	3	25	9	75
Total		45	19	122.6	26	177.4
Mean		15	6.3	40.9	8.7	59.1

Figure 4 shows a bell-shaped curve with a peak around 45 percent for the distribution of percent seed pod development among inflorescences. Curiously, a high number of inflorescences had zero pod development which does not conform to the rest of the distribution curve. Examination of the data indicated several explanations; two plants completed flowering very early and may have missed the peak of pollinator activity, two had damaged inflorescences with only two surviving flowers and two were small late flowering plants.

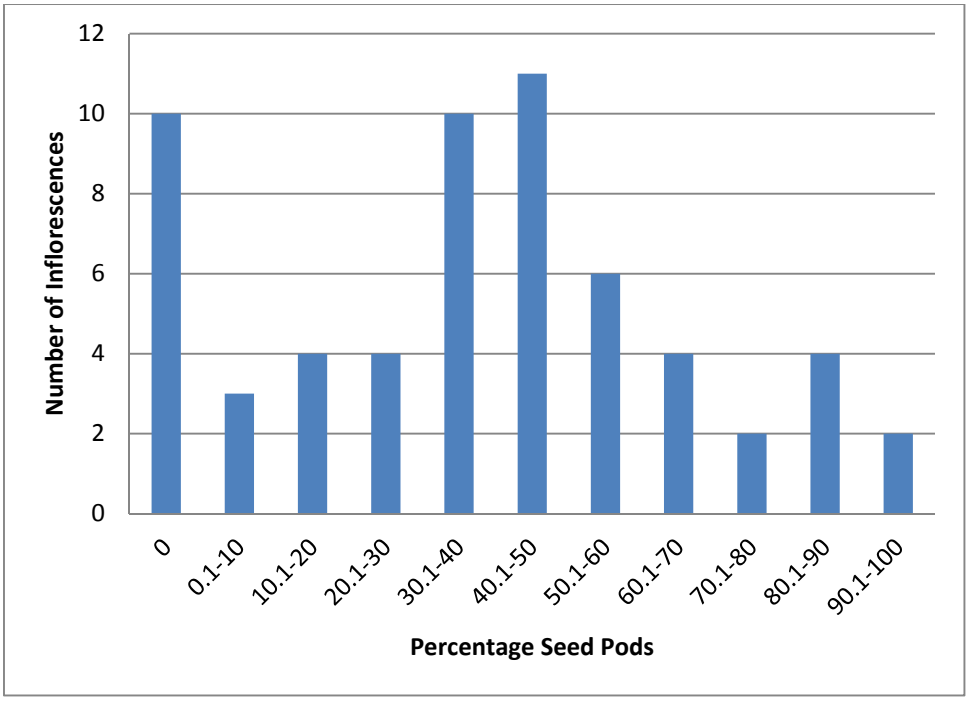

Figure 4. Pollination success of individual inflorescences

DISCUSSION

Genoplesium species present

Two species of *Genoplesium*, *G. littorale* and *G. rufum s.l.*, occur at North Tuncurry. *G. littorale* is by far the more common species comprising approximately 87.5 percent of the population on the study area. The two species are very similar and difficult to distinguish in the field. They differ principally in the possession of small sepal glands in *G. rufum s.l.* and subtle differences in labellum colour and structure. The sepal glands differ in their size and conspicuousness among *G. rufum s.l.* plants and labellum structure is very difficult to see in the field without harming the flower.

Previous work on the study area has assumed the presence of only one *Genoplesium* species, *G. littorale*, until the discovery of *G. rufum s.l.* by Isaac Mamott in 2012. The fact that *G. rufum s.l.* comprises approximately 12.5 percent of the population is significant and has implications for management of *G. littorale* and the interpretation of the results of this study.

Survival of inflorescences

Marking and monitoring of individual plants allowed estimates to be made of inflorescence survival. This showed that less than half of the inflorescences survived from flowering to seed pod maturity. Most inflorescences were lost to herbivory, probably by macropods. Losses were

greatest in areas with dense shrub cover favoured by small wallabies and least in open areas, or counter-intuitively, close to a main road, firebreak and residences, where adverse influences due to humans might be expected. The proximity of human activity may have reduced macropod use of the area.

Pollination mechanism of *G. littorale*

The observations of floral morphology, pollinaria removal and pollen deposition in *G. littorale* and *G. rufum s.l.* are all consistent with insect-mediated pollination. The evidence does not support the existence of autogamy or apomixy in *G. littorale*. If *G. littorale* was obligately autogamous, all flowers developing seed pods would have retained their pollinaria and a mechanism would be present for transfer of pollen onto the stigma. No such mechanism is present. If the species was facultatively autogamous, flowers from which pollinaria had not been removed by insects and which had not been pollinated by insects, would have a mechanism for transfer of pollen from the anthers to the stigma. No evidence for such transfer was found. Similarly, no evidence exists for apomixy, or development of seed without fertilisation. In addition, if apomixy or self-pollination occurred, nearly all flowers would develop seed pods, which is not the case.

Pollination success

The pollination levels found in this study compare very favourably with those recorded in orchids generally, which are often quite low. The low levels of pollination in most orchids are compensated by the very high numbers of seeds produced per capsule, which is likely to be several hundred per pod in *Genoplesium*. The high levels of seed set in *G. littorale* are reflected across the study area in large healthy populations of juvenile plants. Many mature flowering plants on the study area were surrounded by groups of seedlings developed from seed shed in previous years. It is clear the population is actively regenerating and increasing in size.

Hybridisation?

Some results in this study suggested hybridisation may be occurring between *G. littorale* and *G. rufum s.l.* The two species occur in close proximity, often intermingled, potentially facilitating pollen transfer, even by small flies that are unlikely to move long distances. Given that multiple species of chloropids are attracted to *G. littorale* and that one of these was confirmed to be attracted to *G. rufum s.l.*, insect-mediated hybridisation is a strong possibility. This is particularly so in food reward or food deception systems where each species has multiple pollinators that may be shared.

In addition, binocular microscope examination of flowers from 41 plants suggested some had characteristics intermediate between *G. littorale* and *G. rufum s.l.* Some plants with sepal glands (*G. rufum s.l.*) had labellums more like *G. littorale*. Conversely, some plants lacking sepal glands had labellums similar to *G. rufum s.l.*. However, these results are provisional, given the difficulty of identifying labellum features in alcohol preserved specimens. Conclusive resolution of this problem requires examination of fresh flowers and/or genetic analysis. This is an important issue for *G. littorale* which risks losing its genetic integrity if extensive hybridisation with *G. rufum s.l.* is occurring.

Pollinators

In agreement with previous observations on other species of *Genoplesium* (Bower, 2001a), this study found the pollinators of *G. littorale* are tiny flies of the family Chloropidae. Dr D. Bickel (Australian Museum, Sydney) considers there are five species among the flies collected. Three of these, *Cadrema* sp. 1, *Conioscinella* sp. 1 and *Conioscinella* sp. 3 carried *Genoplesium* pollinaria on the centre of the thorax and are confirmed pollinators. Two other species caught in low numbers, *Conioscinella* sp. 2 and *Conioscinella* sp. 4, lacked pollinaria, but nevertheless are

potential pollinators. Pollinators were caught throughout the study area with the dominant pollinator, *Conioscinella* sp. 1, being caught in all four sub-populations sampled. The prevalence of pollinators is confirmed by the successful pollination of *G. littorale* in all four sub-populations.

According to Colless and McAlpine (1991), the adults of Chloropidae '*are of almost ubiquitous occurrence, and the larvae inhabit a wide range of habitats, though still little known*'. It is evident that the chloropid pollinators of *G. littorale* are common at North Tuncurry, especially given the very high pollination percentages that occur on some plants. Such high pollination levels are likely to be achieved when inflorescences at the peak of their attractiveness coincide with favourable weather conditions for chloropid activity. These are sunny days with temperatures in the high 20s and high relative humidity. Such conditions occurred on March 12 to 14 when this study was undertaken. Over two days of collection, in excess of 50 observations of pollinators on plants were made, on occasions with individuals of two different species on the same plant. *G. littorale* is stimulated to flower by high rainfall in late summer and early autumn, which is also likely to stimulate emergence of adult chloropids, thereby achieving synchrony between plant and pollinator.

The specific relationship between outcrossing *Genoplesium* species and pollinators in the related fly families Chloropidae and Milichiidae is unusual, especially for an orchid genus with species providing nectar rewards for pollinators. Generally, when nectar is available, it is exploited by a diverse range of insects from several insect orders [e.g. Hymenoptera (wasps, bees and ants), Diptera (flies), Coleoptera (beetles), Lepidoptera (butterflies and moths)] and many families and genera within them. This is true of the nectar-rewarding orchid genus *Prasophyllum*, which is closely related to *Genoplesium* (Bower, 2001b). However, although there is a specific relationship between the genus *Genoplesium* and the families Chloropidae and Milichiidae, pollinator specificity is lacking at the species level in *G. littorale*, in that it clearly attracts multiple chloropid species as pollinators. *G. littorale* is only the second *Genoplesium* species clearly shown to attract multiple chloropid or milichiid fly species. Bower (2001a) reported that flies collected on *G*. aff. *rufum* by A. Logan included two chloropid species and a milichiid. However, the ability of these flies to effect pollination was not determined in that case.

Food Deception?

Several insect pollinated *Genoplesium* species secrete abundant nectar from the labellum callus (Bower, 2001a). This was noted for *G. rufum* in this study, but no nectar was observable in flowers of *G. littorale*. Some *Genoplesium* species are also known to emit food odours detectable by humans, but others appear odourless to the human nose (Bower, 2001a). The lack of observable nectar in *G. littorale* suggests it offers little or no reward to pollinators, indicating it may be one of the many food-deceptive species in the orchid family. However, more detailed studies are needed to test this hypothesis. Deceptive orchids usually have much lower levels of insect visitation than rewarding orchids because deceived insects learn to avoid false stimuli. *G. littorale* had relatively low levels of pollinator activity in this study compared with reports of some *Genoplesium* species attracting swarms of flies to fresh flowers (Garnet, 1940; Bates, 1981, 1988). However, against this idea is the fact that swarms of pollinators were not seen on *G. rufum*, which has abundant nectar, during this study. If *G. littorale* is food-deceptive it would be the first example in *Genoplesium*.

Ecology of Chloropids

G. littorale, and indeed all outcrossing *Genoplesium* species for which pollinators have been collected, are dependent on chloropids and/or milichiids for pollination. The biological basis of the specific relationship is unknown. It has generally been assumed that *Genoplesium* attracts chloropids with food odours and a nectar reward. However, the specificity of the attraction suggests that *Genoplesium* flowers are offering a specialised reward uniquely attractive to

chloropids and milichiids. The following review summarises the known biology of the genus *Conioscinella* and the family Milichiidae to gain an insight into the possible stimuli being offered by *Genoplesium*.

The swarming behaviour of chloropids reported on some *Genoplesium* species is reminiscent of the behaviour of 'kleptoparasitic' chloropids and milichiids that are attracted to the dying or recently killed insect prey of spiders, assassin bugs, robber flies and other arthropod predators. Chloropids and milichiids may respond rapidly and in numbers to the food of predatory insects, whereupon they feed on the leaking hemolymph (blood) of the victim, effectively stealing food from the much larger predator. Many species of milichiids and chloropids, including several *Conioscinella* species, are known to feed in this way. Sivinski *et al*. (1999) summarise the literature on this phenomenon, citing 12 species of milichiids and seven species of chloropids, including a *Conioscinella* species, as kleptoparasites of predatory arthropods. Robinson and Robinson (1977) also record kleptoparasitism by *Conioscinella* flies on the cricket prey of an orb spider.

Some milichiids rest passively on the thorax of large spiders, such as *Nephila* spp., waiting for them to capture prey (Robinson and Robinson, 1977). Chloropids are also known to hitch rides on the thorax of Robber Flies (Asilidae) so as to be first thieves on the scene of a kill. Excellent photographs of asilids with hitch-hiker chloropids can be seen at:

http://diptera.myspecies.info/category/diptera-classification/chloropidae, and
http://www.christinakwapich.com/?_escaped_fragment_=media/ch6q.

As soon as the prey hemolymph starts to exude from the wounds, the hitch hikers cluster on the victim and imbibe the fluids, even as the larger predator continues to manipulate and consume the prey.

Most milichiid and chloropid kleptoparasites are not hitch-hikers, but nevertheless appear very rapidly on freshly killed prey (Robinson and Robinson, 1977; Eisner *et al*. 1991). The mass attraction of chloropids and milichiids to the herbivorous bug prey of Assassin Bugs is illustrated in the following website photos:

http://beetlesinthebush.wordpress.com/category/arthropoda/insecta/diptera/chloropidae/, and
http://www.alexanderwild.com/keyword/assassin%20bug/1491457998_qT2GxTz#!i=1491457998&k=qT2GxTz.

Milichiid flies invariably approach prey from downwind indicating they are following an odour trail (Eisner *et al*., 1991). They feed as soon as landing, rapidly gorging until their abdomens become grossly distended (Eisner *et al*., 1991).

The flies respond to volatile defensive chemicals emitted by Heteropteran prey and possibly other released volatiles. One milichiid and fifteen chloropid species were caught in traps baited with volatile defensive and pheromonal compounds [(*E*)-2-hexenal, (*E*)-2-octenal and (*E*)-decenal)] produced by Heteroptera under attack (Aldrich & Barros, 1995). Zhang and Aldrich (2004) attracted large numbers of chloropid flies of four species, including two *Conioscinella* species, and a milichiid to hexyl butyrate and (*E*)-2-hexenyl butyrate found in the metathoracic scent glands of plant bugs (Heteroptera: Miridae). Zhang and Aldrich (2004) concluded that '*chloropid and milichiid flies use volatile defensive and pheromonal compounds from plant bugs to find freshly injured or dead bugs on which to feed*'. Zhang and Aldrich (2004) also suggest that since chloropids and milichiids are known to respond to a wide range of arthropod prey including bees and crickets, that there are likely to be many more insect volatiles to which various species respond.

Interestingly, females vastly predominate in all collections of chloropids and milchiids from arthropod prey and chemical bait traps (Robinson and Robinson, 1977; Sivinski, 1985; Eisner *et*

al., 1991; Aldrich and Barros, 1995; Zhang and Aldrich, 2004). The almost exclusive attraction of females suggests they need a protein rich meal for egg maturation as demonstrated for anautogenous mosquitoes (Eisner *et al*. 1991; Zhang and Aldrich, 2004), and a common requirement of many Diptera. The need for a protein meal, and the short time that it is likely to be available, explain the urgency of female responses to newly captured prey.

A Prey Mimicry Pollination Syndrome in *Genoplesium*?

Females greatly dominated the catches of *Conioscinella* species on *G. littorale* (Table 4), which suggests that *Genoplesium* species specifically attract kleptoparasitic chloropids and milichiids for pollination. Genoplesiums may mimic the odours emitted by particular struggling arthropod prey. It is also possible that the nectar of *G. rufum s.l.* and other nectariferous *Genoplesium* species mimics some key properties of insect hemolymph, rather than simply containing the high glucose, sucrose or fructose levels characteristic of nectar in most flowers. It may be that *Genoplesium* 'nectar' is in fact a 'pseudo-hemolymph' and that *Genoplesium* species are arthropod prey mimics. Reports of swarms of chloropids around some *Genoplesium* species (Bower, 2001a) appear to represent similar behaviour to that observed in chloropids around arthropod predators and their prey. Prey mimicry may also explain the apparent lack of nectar in *G. littorale*, which may be exploiting the urgent drive in kleptoparasitic female chloropids and milichiids to find dying insects. Even in the absence of hemolymph, or pseudo-hemolymph, chloropids may enter the flowers looking for prey. This hypothesis is supported by the lack of references in the literature to female chloropids feeding on the nectar of flowering plants other than *Genoplesium*.

The above new hypothesis of a prey mimicry pollination syndrome in *Genoplesium* is considered to best fit the available information on the biology of *Genoplesium* and its pollinators. Although prey mimicry is a new pollination syndrome for Australian orchids, it has been demonstrated in several orchid genera in the northern hemisphere. *Epipactis helleborine* is primarily pollinated by social wasps, such as *Vespula vulgaris* and *V. germanica*, which it attracts by emitting odours, 'green-leaf volatiles', released by plants under attack by herbivores such as caterpillars (Brodman *et al.*, 2008). Parasitic wasps use the volatiles emitted by damaged plants to find their caterpillar prey. The rewardless orchid *Dendrobium sinense* is pollinated by a Hornet, *Vespa bicolor* that attacks a red patch on the centre of the labellum. *V. bicolor* is attracted to the flowers of *D. sinense* by odours mimicking the alarm pheromones of Asian Honeybees (*Apis cerana*) of which *V. bicolor* is a predator (Brodman *et al*., 2009). The terrestrial orchid *Epipactis veratrifolia* is pollinated by several species of Hoverflies whose larvae feed on aphids. The hoverflies are attracted to the orchid by odours mimicking the alarm pheromones of some aphids and lay eggs on aphid like bumps on the flowers (Stokl *et al*., 2011). Clearly, the hypothesised *Genoplesium* / Chloropid prey mimicry pollination mechanism is but one of a number of bizarre modes of prey mimicry in orchids.

Conserving pollinators – General considerations

Conservation biology is a relatively new science and it is fair to say that many of the questions needing answers have only just begun to be addressed in the scientific literature. A great deal of controversy and uncertainty surrounds some issues and in other cases information is lacking entirely. This is especially the case for conservation of plants and their insect pollinators (Packer and Owen, 2001). Cane and Tepedino (2001) noted there had been little attempt to rigorously address the key issues for any plant/pollinator pairing in nature and this remains the case. There are no generalisations on which to confidently predict what might be the minimum effective population sizes or living areas required by populations of plants and their pollinators for long term viability.

Before a reasonably reliable assessment of the long term habitat needs of *G. littorale* and its pollinators can be made, it would be necessary to determine the minimum effective population size (N_e) of each. This is not an easy task. Effective population size refers to the number of

reproductive units needed to ensure long term viability of populations. It is affected by many variables and is not necessarily the same as the number of plants or female pollinators in the population as determined by a simple census. Among the variables affecting N_e are:

- The number of generations for which survival is required (i.e. 50, 100, 500 years etc). The longer the time period required, the higher the minimum effective population (Packer and Owen, 2001).

- Life history attributes such as sex ratio, variance in numbers of offspring and haplodiploid versus diploid-diploid mating systems among others.

- Habitat diversity, particularly the presence of refuge areas that allow survival during periods of high environmental stress.

- Population variability due to factors such as the direct effects of climate and fire, and indirect effects on pollinator food supplies (adults) and larval food.

- Genetic effects in low populations such as declines in heterozygosity (inbreeding), and bottlenecks as a result of reductions to very low numbers. Genetic modelling (Packer and Owen 2001) indicates that population sizes of less than 100 are very prone to long term extinction due to declines in heterozygosity resulting from inbreeding.

- Capacity for immigration, i.e. metapopulation structure, dispersal behaviour, habitat connectedness and ability to recolonise after local extinction (Cane, 2001).

Such information is lacking for *G. littorale* and all other *Genoplesium* species and their pollinators. It would require a series of very large studies to determine these variables with any certainty. Such studies would require specialist expertise in several fields and would be expensive. Clearly this is impractical in the situation of *G. littorale* at Tuncurry, and in fact, has not been achieved for any plant and its pollinators. The only insect for which such comprehensive information is available appears to be the Bay Checkerspot Butterfly, *Euphydryas editha bayensis*, in California.

There are very few estimates in the literature of minimum viable population size or minimum viable habitat area for insects. Erhlich and Murphy (1987) estimated that 25 ha may be sufficient for long term survival of *E. editha bayensis* in isolated serpentine outcrops in California, provided the habitat includes refugia from environmental extremes. Subsequently, following a detailed analysis of the population dynamics of two smaller populations of *E. editha bayensis*, 2.6 and 9.8 ha, that became extinct, Hellmann *et al* (2003) considered that a 25 ha population was also at risk of extinction. The more stable dynamics of a 100 ha *E. editha bayensis* population led Hellmann *et al*. (2003) to conclude that 100 ha or more may be sufficient for medium to long term survival of this species in the absence of substantial climate change.

Main (1987) considered that 35 ha of suitable habitat would maintain two large mygalomorph spiders in Western Australian wheatbelt remnants. However, this estimate was based on the persistence of the species in remnant landscapes (granite outcrops) that had been isolated for millennia, rather than on a long term ecological study.

Biedermann (2000) concluded from a metapopulation study of a network 506 small host plant patches that the froghopper, *Neophilaenus albipennis*, could persist long term in an area of 6 to 12 ha depending on metapopulation structure. Similarly, Jones *et al*. (2008) concluded from detailed sampling of an isolated tropical montane forest remnant in Mexico that several species of rare weevils (Curculionidae) can maintain viable populations in areas of less than 10 ha. Unfortunately, there do not appear to be any estimates of minimum viable population size or habitat area for any Chloropids or other small Diptera.

Conservation of *G. littorale* and its *Chloropid* pollinators

This section uses the limited available information on area requirements for insect conservation and the biology of chloropids to assess the likely areas needed to conserve viable populations of the pollinators of *G. littorale* in the long term. There are a number of relevant aspects to consider.

- ***Size of the pollinators***

The pollinators of *Genoplesium* are very small flies, so small they can move through insect mesh screen doors. Insects of this size seem unlikely to require large areas in order to maintain viable populations. Since areas in the vicinity of 25 to 100 hectares have been recommended for some of the larger invertebrates, it is reasonable to conclude that insects as small as chloropoids may be able to maintain viable populations in smaller areas. However, this depends on the availability of resources essential to their survival. Accordingly, it is necessary to consider the biology and ecology of chloropids when estimating their habitat requirements (see below).

- ***Diversity of pollinators***

G. littorale attracts multiple chloropid species as pollinators. The existence of multiple pollinators has a number of likely implications.

  - Given the limited sampling in this study it is likely that other potential pollinator species may occur at North Tuncurry.

  - The dominant pollinators may change from season to season depending on the weather and the ecology of different fly species. The dominant pollinators may also switch in response to habitat changes, such as fire or other disturbances, and through different stages of vegetation succession following disturbance.

  - The existence of multiple pollinators is therefore likely to result in more stable pollination between sites and years than would be the case for orchid species with a single pollinator.

  - According to Colless and McAlpine (1991) chloropids '*are of almost ubiquitous occurrence*', suggesting that at least some species are present in all environments. The observations of Jones (1970) and Bates (1981) indicate that chloropids capable of pollinating several *Genoplesium* species occur in urban areas. This suggests that some chloropids can persist in extremely disturbed situations. Accordingly, it is considered likely that some, if not all, of the pollinators of *G. littorale* would persist in bushland remnants within a predominantly urban setting.

- ***Resource requirements and minimum viable area***

If the hypothesis of prey mimicry in *G. littorale* is true, as seems likely, the key ecological requirements for survival of its *Conioscinella* pollinators are larger arthropods, their arthropod predators and the habitats that support them. Kleptoparasitic chloropids are part of, and depend on, the arthropod food web within the ecosystems they inhabit. Consequently, conservation of kleptoparasitic chloropids requires the presence of a functioning food web able to support medium to large arthropod predators. Unfortunately, there appear to be no studies on minimum viable population sizes or habitat areas for such predators, although it is well recognised that mammalian and avian predators require significantly larger foraging areas than herbivores. Consequently, minimum viable areas for arthropod predators are likely to exceed estimates for other medium to large non-predatory arthropods, i.e. larger than 25 to

100 hectares. Maintenance of sufficient arthropod predator diversity may require 200 ha or more in isolated vegetation patches. However, if sufficiently wide corridors of natural vegetation are maintained between sub-populations of *G. littorale* and large bushland reserves, immigration of arthropods could be expected to allow recolonisation of smaller areas following catastrophic events such as wildfire, or localised stochastic extinctions.

- ***Conservation of G. littorale and its pollinators at North Tuncurry***

The northern population of *G. littorale* (represented by Groups B, C and D in this study) on the eastern side of the Tuncurry Waste Management Centre are considered to be secure in the long term. These are also the largest known populations of the species. The exclusion of 242 hectares of habitat from the development to the south and east of this population provides sufficient habitat to maintain the arthropod food webs on which the chloropid pollinators of *G. littorale* depend, especially given the shared boundary with Darawank Nature Reserve (575 ha) to the north.

The southern populations of *G. littorale* (including Group A in this study) occur within a relatively narrow corridor between The Lakes Way and the Notional Development Footprint (NDF). The width of this corridor has varied with different versions of the NDF, such that the area within the corridor was approximately 25 ha as at December 2012 with a narrow neck in the middle. The size of this corridor is considered to be too small to guarantee sufficient arthropod diversity to support the existing suite of *G. littorale* pollinators in the medium to long term. In addition the narrow neck presents a potential bottleneck to arthropod re-establishment from population reservoirs to the north in the event of local extinctions. Consequently, in discussions with Landcom the NDF western boundary has been moved east to approximately double the corridor area to 50 ha and remove the bottleneck. It is considered that this will both substantially increase the stability of existing pollinator populations in the corridor and provide a more effective linkage to bushland reserves to the north.

CONCLUSIONS

1. Two species of *Genoplesium* occur at North Tuncurry, the Tuncurry Midge Orchid, *G. littorale* and the Red Midge Orchid, *G. rufum s.l*. Examination of a random sample of 34 single flowers and six inflorescences revealed that *G. rufum s.l.* comprises approximately 12.5 percent of the population.

2. Microscope examination of flowers on seven preserved inflorescences of *G. littorale*, and three of *G. rufum s.l.*, showed:

    a. They had high levels of pollinaria removal by insects, 28 to 90 percent in *G. littorale* and zero to 44 percent in *G. rufum s.l.*

    b. Pollination of stigmas varied from 15 to 79 percent in *G. littorale* and zero to 40 percent in *G. rufum s.l.*.

    c. Swelling of ovaries was evident in 15 to 68 percent of *G. littorale* flowers and zero to 6 percent in *G. rufum s.l.*

3. Observations of floral morphology, pollinaria removal and pollen deposition in *G. littorale* and *G. rufum s.l.* are consistent with insect-mediated pollination in both species.

4. The evidence does not support the existence of self-pollination (autogamy) or apomixy in *G. littorale* or *G. rufum s.l.* By contrast, flowers of *G. rufum s.l.* exhibited strong nectar secretions on the labellum.

5. No odour was detectable by smelling *G. littorale* inflorescences in warm conditions, nor was any nectar secretion detected on the labellum. The apparent lack of nectar secretions by *G. littorale* suggests it may be a food deceptive species.

6. Twenty two small flies were captured on *Genoplesium* inflorescences; 10 had *Genoplesium* pollinaria adhering to the centre of the dorsal thorax.

7. The flies comprised five species in the family Chloropidae; one *Cadrema* species and four *Conioscinella* species. The two *Cadrema* specimens, five specimens of *Conioscinella* sp. 1 and three specimens of *Conioscinella* sp. 2 carried *Genoplesium* pollinaria and are confirmed pollinators of *G. littorale*. One specimen of *Conioscinella* sp. 1 was captured on *G. rufum* indicating at least one of the pollinators visits both orchid species.

8. Examination of marked plants on 23 April showed that less than half (46 percent) had survived. Most losses are attributable to macropod grazing.

9. Seed pod set on individual plants varied from zero to 100 percent with a mean of 42.6 percent over the whole population. Seed pod set varied across the study area suggesting pollinator populations also varied. Seed set is relatively high in *G. littorale* compared with many other orchids. This and the presence of many seedling plants, indicate the population is actively reproducing and expanding.

10. There is some evidence that hybridisation between *G. littorale* and *G. rufum s.l.* may be occurring. They appear to share at least one of the pollinators and some individuals with intermediate character traits were found by detailed flower examinations.

11. The *Conioscinella* pollinators of *G. littorale* belong to a group that includes many species of 'kleptoparasites' that are attracted to the newly captured prey of arthropod predators. Kleptoparasites feed on the leaking hemolymph of dying or recently killed insects. It is likely that the pollination strategy of *Genoplesium* is prey mimicry in which kleptoparasitic chloropids are attracted to flowers by odours that mimic those released by struggling insect prey. This is a new hypothesis for pollination in *Genoplesium*. However, it fits the known facts of *Conioscinella* biology and explains the pollination specificity between *Genoplesium* and chloropids.

12. Conservation of kleptoparasitic chloropids is likely to depend on the continued presence of viable populations of medium to large insects and their arthropod predators. Minimum viable areas for arthropod predator conservation are unknown, but may exceed 200 ha. It is considered that the area proposed to be set aside to the north of the development is sufficient to maintain the arthropod diversity on which the northern *G. littorale* pollinator populations depend. *G. littorale* pollinator populations in the western corridor between The Lakes Way and the NDF may be less stable. However, it is considered that the revised area of 50 ha (as at July 2013) significantly increases the stability of pollinator populations in the corridor and provides an adequate linkage for insect dispersal from the north.

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ATTACHMENT 1

Assessment of seed pod set, 23 April, 2013

Appendix 1. Assessment of seed pod set, 23 April, 2013

Group	Plant No.	Fate	Total flowers	Pods		Unpollinated flowers		Comment
				No.	%	No.	%	
A	1	M						
A	2		8	4	50	4	50	
A	3		13	1	7.7	12	92.3	Snapped off near base
A	4		2	0	0	2	100	Rest of inflorescence gone
A	5	H						
A	6	M						
A	7	M						
A	8	M						
A	9	M						
A	10		14	0	0	14	100	
A	11		14	0	0	14	100	
A	12		12	3	25	9	75	*G. rufum s.l.* (I Mamott)
A	13		8	0	0	8	100	
A	14		10	2	20	8	80	
A	15		6	0	0	6	100	
A	16	H						
A	17		7	3	42.9	4	57.1	
A	18		7	2	28.6	5	71.4	Pulled out and lying on ground
A	18a	S						
A	19	S						*G. rufum s.l.*
A	20	Sh						
A	21							
A	22		16	9	56.3	7	43.8	Broken off at base, lying on ground
A	23		9	8	88.9	1	11.1	Stem broken nr top, able to mature?
A	24		5	0	0	5	100	
A	25	H						
A	26	H						
A	27		9	3	33.3	6	66.7	
A	28		16	7	43.8	9	56.3	
A	29		7	1	14.3	6	85.7	
A	30		12	6	50	6	50	*G. rufum s.l.*
A	31		15	6	40	9	60	
A	32		14	3	21.4	11	78.6	
A	33		11	7	63.6	4	36.4	
Total			**215**	**65**	**585.8**	**150**	**1514.4**	
Mean			**10.2**	**3.1**	**27.9**	**7.1**	**72.1**	
B	1	H						
B	2	S						
B	3	M						
B	4	H						

Group	Plant No.	Fate	Total flowers	Pods		Unpollinated flowers		Comment
				No.	%	No.	%	
B	5	M						
B	6	H						
B	7	M						
B	8	Sh						Inflorescence shrivelled
B	9	H						
B	10	H						
B	11	H						
B	12							
B	13	H						
B	14	H						
B	15	H						
B	16							
B	17		21	10	47.6	11	52.4	*G. rufum s.l.*
B	18	H						
B	19							
B	20		22	15	68.2	7	9.1	
Total			**43**	**25**	**115.8**	**18**	**61.5**	
Mean			**21.5**	**12.5**	**57.9**	**9**	**30.8**	
C	1	M						
C	2	S						
C	3		22	2	9.1	19		
C	4		11	6	54.5	5		
C	5	M						
C	6		14	5	35.7	9	64.3	
C	7	M						
C	8	M						
C	9	Sh						Shrivelled head
C	10	M						
C	11	M						*G. rufum s.l.*
C	12	H						
C	13	H						
C	14	M						
C	15	M						
C	16	M						
C	17	M						
C	18							Not found
C	19							Not found
C	20							Not found
C	21	M						
C	22	M						
C	23		15	9	60	6	40	

Group	Plant No.	Fate	Total flowers	Pods		Unpollinated flowers		Comment
				No.	%	No.	%	
C	24		11	4	36.4	7	63.6	
C	25	M						Tag pulled out, no plant
C	26	S						
C	27	H						
C	28		9	3	33.3	6	66.7	
C	29		9	8	88.9	1	11.1	
C	30	S						*G. rufum s.l.*
C	31		8	5	62.5	3	37.5	
C	32	H						
C	33	H						
C	34		6	1	16.7	5	83.3	
C	35		13	2	15.4	11	84.6	
C	36	H						
C	37	M						
C	38		10	0	0	10	100	
C	39	H						
C	40	M						
C	41		13	5	38.5	8	61.5	
C	42		2	0	0	2	100	Lost rest of inflorescence
C	43		12	5	41.7	7	58.3	
C	44	H						
C	45	M						Tag pulled out, no plant
C	46	H						
C	47	H						
C	48	H						
C	49	H						
C	50	H						
C	51		8	0	0	8	100	
C	52	M						
C	53	M						
C	54		25	9	36	16	64	
C	55		10	5	50	5	50	
C	56	Sh						Shrivelled head
C	57		14	5	35.7	9	64.3	
Total			**212**	**74**	**614.4**	**137**	**1049.2**	
Mean			**11.8**	**4.1**	**34.1**	**7.6**	**58.3**	
D	1		14	5	35.7	9	64.3	12 Shrivelled buds
D	2	Sh						Shrivelled, fallen
D	3		16	6	37.5	10	62.5	
D	4		12	7	58.3	5	41.7	
D	5		12	5	41.7	7	58.3	

Group	Plant No.	Fate	Total flowers	Pods		Unpollinated flowers		Comment
				No.	%	No.	%	
D	6		12	11	91.7	1	8.3	
D	7		9	4	44.4	5	55.6	
D	8		6	5	83.3	1	16.7	
D	9		14	11	78.6	3	21.4	
D	10		10	7	70	3	30	
D	11		17	10	58.8	7	41.2	
D	12		12	1	8.3	11	91.7	
D	13	H						
D	14	H						
D	15	H						
D	16		15	7	46.7	8	53.3	
D	17		13	11	84.6	2	15.4	
D	18	Sh						Knocked over, shrivelled
D	19		0	0	0	0	0	
D	20		17	13	76.5	4	23.5	
D	21	M						
D	22		12	3	25	9	75	*G. rufum s.l.*
D	23	M						
D	24		9	6	50	3	25	3 damaged flowers
D	25	S						
D	26	H						
D	27		11	6	54.5	5	45.5	
D	28		14	14	100	0	0	
D	29	M						
D	30	H						
Total			**225**	**132**	**1045.6**	**93**	**729.4**	
Mean			**11.8**	**6.9**	**55**	**4.9**	**38.4**	
M: Missing; H: Herbivory; S: Sampled; Sh: Shrivelled								