

**POLLINATION OF THE TUNCURRY MIDGE ORCHID
(*Genoplesium littorale*)**

SUPPLEMENTARY INFORMATION

JUNE 2014

INTRODUCTION

FloraSearch (2013) reported to UrbanGrowth NSW on the pollination of the Tuncurry Midge Orchid (*Genoplesium littorale*) (TMO), which is listed as Critically Endangered at both the NSW and Commonwealth levels. TMO occurs on land north of Tuncurry that is proposed for a housing subdivision by UrbanGrowth.

A large field survey effort has been made since 2008 (Paget, 2008; RPS, 2012) to determine the numbers and distribution of TMO in the Forster – Tuncurry area and surrounds. In the course of this work, Isaac Mamott of RPS discovered plants that appeared to be a second species of *Genoplesium* within the populations north of Tuncurry. These plants were distinguished by possessing globular white glands on their lateral sepals. Such glands are absent from the great majority of plants in the population. Specimens of the plants with glands submitted to the Royal Botanic Gardens and Domain Trust Sydney were identified as *Genoplesium rufum* (Red Midge Orchid) (RMO) by Dr Peter Weston (Isaac Mamott, pers. comm.).

The presence of a second, subtly different *Genoplesium* species at North Tuncurry greatly complicated the pollinator research and the interpretation of the results (see discussion in FloraSearch, 2013). An examination of the taxonomic literature on TMO, RMO and close relatives indicated the presence of sepal glands is not used as a diagnostic feature by Jones (2006). Because the presence of sepal glands appears to lack diagnostic usefulness, Florasearch (2013) considered that labellum characters are more likely to be diagnostic. Indeed, FloraSearch (2012) suggested that TMO might be separated from RMO at Tuncurry on the basis of differences in the shape and sheen of cells in the groove of the labellum callus. Unfortunately, the labellum is hidden deep within the flower and cannot be examined closely in the field without damaging the plant.

Accordingly, in 2013, FloraSearch collected a sample of single flowers from 41 inflorescences for later microscope examination. The flowers were preserved in alcohol which regrettably leached out the colour making it impossible to use colour-based characters. The lack of colour also made it difficult to see changes in texture and sheen. Consequently, the identifications were somewhat equivocal; no labellum or other characters were found that correlated with the presence or absence of sepal glands (FloraSearch, 2013). An interesting finding among the preserved flowers was variation in sepal gland size, which ranged from large globular glands, that were easily visible in the field, to small inconspicuous glands that were not detected in the field.

In order to overcome the identification problems associated with preservation of flowers in alcohol in 2013, the population was resampled in 2014. [Note: This work was carried out independently of UrbanGrowth NSW, i.e. it was not commissioned by Urban Growth NSW. It was conducted because the author wishes to publish a scientific paper on the pollination of TMO, and in order to do that it is necessary to resolve the uncertainties remaining after the work carried out in 2012 and 2013.]

METHODS

A total of 29 flowers, divided among areas A, B and C, were collected in mid-March, 2014. The flowers were placed in individual labelled vials and stored in a refrigerator at 4 degrees Centigrade. They were examined under a binocular microscope within two days of collection, while still fresh. Each flower was examined in detail with the following features being recorded:

- Lateral sepal – presence and size of apical glands; presence and size of basal hump; width.
- Dorsal sepal – depth; flexure of apex.
- Petal – shape (lanceolate or ovate); apex (acute or acuminate).
- Labellum
 - Groove – shiny or dull; callus ridges (flat or rounded)
 - Base – thickness and shape
 - Callus length
 - Callus shape – narrows evenly or constricted
 - Margin – narrows gradually or abruptly; regular, irregular, erose or toothed.
- Other observations

RESULTS

Sepal glands

The detailed examination showed that there was a gradation in sepal gland size from minute to large, as follows:

- Large glands were observed on six flowers and comprised distinct pale globular masses attached by a short stalk to the tip of the sepal. These are easily visible in the field.
- Smaller vestigial glands are ovoid or cylindrical and attached by short stalks. These were seen on six flowers and are unlikely to be observed in the field.
- Smaller still are mucronate glands which simply project as a short cylindrical point from the sepal apex. They comprise a mass of pale coloured cells that are noticeably smaller than the epidermal cells of the sepal itself. These were also recorded on four flowers.
- Three flowers were observed with minute glands that were simply aggregations of paler, small cells at the apex of the sepals.
- Ten flowers had no discernible glandular cells at the sepal apices.

Labellum

Somewhat surprisingly, the labella on all flowers were similar. There was no evidence of variation in the thickness or fleshiness of the labellum base, a characteristic feature of TMO. In addition, the labellum groove in all but two flowers was recorded as shiny; in one that was pigmented reddish the sheen was hard to see and the other was recorded as 'somewhat shiny'. The labellum callus was also similar in all flowers, extending right to the labellum apex with the groove ending well before the labellum bend in all flowers. The labellum margin was irregular in all but one flower in which the margin was slightly toothed. The labellum narrowed gradually from base to tip in all but six flowers; in three it was a little wider about the middle and in three it narrowed a little abruptly going onto the tip. The shape of the callus plate varied more than the other features; in seventeen flowers it narrowed evenly, but in 12 flowers it either narrowed irregularly (1 flower) or was more or less constricted (11 flowers). However, none of the labellum characters was correlated with the presence, absence or size of the sepal glands.

Other floral segments

Minor variation occurred in the width of the lateral sepals, depth of the dorsal sepal and the flexure of its apex, and the shape and acuteness of the petals, none of which correlated with the presence, absence or size of the sepal glands.

DISCUSSION

The uniformity of the labella across all 29 flowers strongly suggests there is only a single species represented in the populations examined, i.e. TMO. The data indicate that TMO may have sepal glands of various sizes. Only the largest glands can be easily observed in the field (when you are looking for them). In all other respects, plants with and without obvious sepal glands are similar, which explains why successive botanists have only identified TMO in the North Tuncurry area, until the chance observation by Isaac Mamott in 2011.

Correspondence with Dr Peter Weston on this issue is attached. He concurs it is unlikely that more than one species occurs in the North Tuncurry population.

It is clear that the presence or absence of sepal glands is not a reliable taxonomic character in *Genoplesium*. It is not used in identification keys and Jones (2006) notes there are species in which sepal glands may sometimes be present. TMO is one of the species in which some individuals may have conspicuous sepal glands, but most do not. It is interesting that gland size is very variable in TMO and the smallest glands can only be seen at 10 to 20 times magnification.

It is now evident that RMO is not present at North Tuncurry and that hybridisation between TMO and RMO is not occurring. This greatly simplifies the interpretation of the pollination results obtained in 2013 (FloraSearch, 2013). An updated and amended report will be produced that reflects the new data and interpretation.

CONCLUSIONS

- Detailed examination of fresh flowers in 2014 has shown there is only one species of *Genoplesium* present at North Tuncurry, the Tuncurry Midge Orchid, *Genoplesium littorale*.
- Rather than representing evidence for the existence of a second *Genoplesium* species, the occasional presence of conspicuous sepal glands is a normal feature of *G. littorale*.

REFERENCES

- FloraSearch (2012). *Preliminary Report on Pollination of the Tuncurry Midge Orchid (Genoplesium littorale)*. Report to Landcom, Forster, NSW.
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- Jones, D.L. (2006). *A complete guide to Native Orchids of Australia including the Island Territories*. Reed New Holland, Sydney
- Paget, A. (2008). *Results of Searches for the Tuncurry Midge Orchid (Genoplesium littorale, syn Coronastylis littoralis)*. Report to the NSW Scientific Committee. Hunter-Central Rivers Catchment Management Authority.
- RPS (2012). *Tuncurry Midge Orchid (Genoplesium littorale syn. Coronastylis littoralis)*, Crown Lands, North Tuncurry, NSW. Report to Landcom NSW. RPS Australia East Pty. Ltd., Broadmeadow, NSW.

Signed:



Principal Consultant Botanist

Email Correspondence with Dr. Peter Weston, Royal Botanic Gardens and Domain Trust, Sydney

From: Peter Weston <Peter.Weston@rbgsyd.nsw.gov.au>
Sent: Monday, March 31, 2014 5:49 PM
To: FloraPhoto Enquiries
Subject: RE: *Corunastylis littoralis*

Dear Col,

I cannot remember why I thought that Isaac's specimens were *Corunastylis rufa* rather than *C. littoralis*. However, the main differences between them according to David Jones' descriptions (his original description of *C. littoralis* in *The Orchadian* and his description of *C. rufa* in *Flora of NSW*) are a slight difference in flower size, with *C. littoralis* being slightly larger (e.g. lateral sepals 3-3.5 mm long in *C. rufa*, 4-4.5 mm long in *C. littoralis*) and a slight difference in labellum shape (apex obtuse to acute in *C. rufa*, acuminate in *C. littoralis*). David did not mention the apical glands on the lateral sepals in his description of *C. rufa* but he does illustrate them as present in his *Flora of NSW* treatment. I gather from the fact that he did not mention them that he thinks their presence or absence is not a very reliable character for distinguishing these species. I agree with you that the existence of sympatric populations of *Corunastylis rufa* and *C. littoralis* seems unlikely. I might well have misidentified Isaac's specimens but I think that your suggestion that *C. littoralis* might be just a geographic variant of *C. rufa* seems more likely. However, I would not go ahead and sink *Corunastylis littoralis* under *C. rufa* without further, more detailed analysis because of its rarity and the possibly irreversible consequences for biodiversity conservation of such a decision.

Cheers, Peter

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From: FloraPhoto Enquiries [mailto:enquiries@floraphoto.com.au]
Sent: Monday, 31 March 2014 4:10 PM
To: Weston Peter
Subject: *Corunastylis littoralis*

Hi Peter,

As you know I've been doing some work on the pollination of *Corunastylis littoralis*, last year as a consultancy, but this year at my own expense to tie up some loose ends for a potential publication.

The issue has arisen as to whether some of the population is in fact *C. rufa*, and if so, how much. I understand you identified samples provided by Isaac Mammot as *C. rufa*, I would imagine largely on the basis of the presence of apical glands on the lateral sepals. I attempted to estimate the proportion of *C. rufa* in the population last year and came up with a figure of approximately 12.5 percent. However, I was using single flowers preserved in 70 percent alcohol and, owing to the leaching of colour from the specimens, had difficulty in assessing potential characters other than the sepal glands.

This year I made another attempt using unpreserved refrigerated single flowers, assessed within two days of picking. The results were quite interesting. Although few flowers had large globular glands, more had rather vestigial glands that were either slightly rounded or almost mucronate. In a few cases there was just an aggregation of lighter coloured cells at the sepal apex. In addition, there were no other characters that correlated with the presence of glands. All flowers had generally similar tepals, including the labellum, which was of similar shape, colour, fleshiness and irregular margins in all flowers. My conclusion is that all plants belong to the same taxon, whether they have sepal glands or not, i.e. *C. littoralis* may have sepal glands. Alternatively, *C. littoralis* is no more than a form of *C. rufa*. I would appreciate your thoughts on this.

I still have the specimens in the fridge, but they are now over a week old and may not last much longer. I was wondering if you would like to see them. I could bring them to Sydney this week or pop them in alcohol for later examination.

Regards,

Col

Colin Bower PhD
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