Life-cycle and natural history of the endangered terrestrial orchid *Genoplesium baueri* in eastern New South Wales

Wendy A. Grimm¹, Peter H. Weston², Anthony Manea¹ and Michelle R. Leishman¹

¹Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, AUSTRALIA
²Honorary Research Associate, Australian Institute of Botanical Science, Royal Botanic Gardens & Domain Trust, Sydney, NSW 2000, AUSTRALIA

**Abstract:** Many aspects of the biology of *Genoplesium baueri* R.Br. (Orchidaceae) an endangered terrestrial orchid endemic to New South Wales, Australia, remain unknown. In this study observations were made on (1) plant morphology; (2) the biology of the fruit and seeds; (3) the biology of the plant tuber and the spatial patterning of emergent plants; (4) the morphology of the pollen and the identity of pollen vectors and (5) to develop a preliminary description of the lifecycle of the orchid, by carrying out annual surveys of two key *Genoplesium baueri* populations in northern Sydney, for a 12-year period and collecting data on fruits, tubers and pollinators opportunistically. Individual plants cannot be tracked from year to year.

We observed that each emergent plant produces a tubular leaf and an inflorescence of 1-15 non-resupinate flowers. Fruit capsules contained numerous tiny seeds, which were slightly larger than seeds of a closely related species, *Corunastylis filiformis*. The papillose surface of mature tubers of *Genoplesium baueri* differed greatly from the smooth, spherical tubers of *Corunastylis fimbriata* and is likely to be the site of orchid mycorrhizae. The occurrence of clumps of closely-packed, variously-sized tubers, each subtending single *Genoplesium baueri* plants, warrants investigation as development by vegetative increase at the tuber level provides one of several possible explanations. The morphology of the pollinarium and evidence of Chloropid fly-mediated pollination was observed. Our better understanding of the natural history and biology of *Genoplesium baueri* will contribute to orchid systematics and help inform management decisions regarding this species to ensure its persistence into the future.

**Key words:** myophily, orchid seed, orchid fruit, orchid tubers, pollen vector, Prasophyllinae

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Introduction

*Genoplesium baueri* R.Br., Brittle Midge Orchid or Bauer’s Midge Orchid, is a rare terrestrial orchid species endemic to the Sydney Basin, New South Wales, Australia. It has been listed in both the state and federal conservation legislation as Endangered, and threatened with extinction due to its highly restricted geographical distribution (Sydney to Jervis Bay) and projected decline in key indicators, including abundance and habitat quality of the species (NSW Scientific Committee 2018; Stephenson 2010).

A recently published phylogenetic tree of the subtribe Prasophyllinae, based on an unpublished analysis of ITS nrDNA sequence data (Clements & Jones 2019), suggests that *Genoplesium baueri* is the sister group to *Corunastylis* Fitzg. plus the newly named *Paraprasmusphyllum* M.A.Clem. & D.L.Jones. Morphological and genetic comparisons of species within the genus *Genoplesium* R.Br. prompted Jones et al. (2002) to transfer all species of *Genoplesium* other than *Genoplesium baueri* into the resurrected genus *Corunastylis* Fitzg., a taxonomic change that has been recognized by some but not all orchid taxonomists (Jones 2006; Backhouse et al. 2019; Clements & Jones 2019; cf. Renner 2019). The original description of *Genoplesium baueri* (Brown 1810; Watts et al. 1997) was brief and lacked detail. Descriptions of the genus *Genoplesium sensu lato* (Jones & Clements 1989; Weston 1993; Jones 1993) cannot be applied to the genus *Genoplesium sensu stricto* (Jones & Clements 1989; Jones 2006) with accuracy. Therefore, it is imperative that we acquire as much knowledge as possible on the biology of this endangered species to inform orchid systematics as well as conservation decisions, in order to manage this species to ensure its survival.

In March 2009, we commenced population monitoring at a previously little-known site for *Genoplesium baueri* in Ku-ring-gai Chase National Park, Mt Colah (KCNP) and at a newly discovered site in Ku-ring-gai Wildflower Garden, St Ives (KWG), both located on the northern outskirts of Sydney. Both of these populations grow in very low fertility soils derived from Hawkesbury Sandstone in low woodland on the Hornsby Plateau (Chapman & Murphy 1989; Carolin & Tindale 1994) and are key to the survival of the species (Saving our Species *Genoplesium baueri* available at https://www.environment.nsw.gov.au/savingourspeciesapp/Project.aspx?results=c&ProfileID=10875). The monitoring of these two populations is ongoing. This report is the culmination of what we learnt about the biology of *Genoplesium baueri* over the twelve years of monitoring these populations until June 2020. Over the course of the population monitoring, we collected data opportunistically on plant structure, pollen vectors and questions pertaining to the apparent sporadic flowering of the species. Regulations restricting sampling and collecting endangered species means that only opportunistic sampling was possible, for example, when tubers were exposed by animal diggings.

Methods

From late December each year, the sites were systematically searched on a weekly basis for *Genoplesium baueri* plants. When a plant was found it was allocated a unique sequential plant code, photographed, plotted onto a site map and its GPS location was recorded. A fine bamboo skewer, 20–30 cm long and bearing codes for year and plant number, was inserted a short depth into the substrate at a distance of 10 cm in a predetermined direction from the base of each inflorescence. Plants were also photographed at each subsequent survey as flower and fruit development progressed. These data were then used to track the plants through time in that growing season. Extensive data on population demographics (Grimm et al. 2020) are published separately.

During the 2010 growing season, an inflorescence with one ripe fruit was submitted as a voucher specimen to the National Herbarium of NSW. Additionally, in 2018, the dimensions of fully-formed green ovaries were measured (to 0.01 mm) in situ using digital callipers. A small sample (<100) of *Genoplesium baueri* seed was collected (May 2018) in situ from a naturally dehisced capsule and the testa (seed coat) and embryo visible inside the seed coats were examined using transmitted light microscopy (at x10, x40, Olympus EH series). Seeds from a closely related taxon, *Corunastylis fitilformis*, were examined for comparison.

Tuber morphology and the relationship between tubers of individual plants within clumps were observed opportunistically in the field when the substrate or the tuber had been excavated by foraging animals. The maximum height, width and thickness of tubers were recorded. A single tuber of *Genoplesium baueri* was dissected before being fixed in FAA solution (90 mL 70% ethanol, 5 mL acetic acid, 5 mL formalin) for eight days, then washed in water and 70% ethanol. The tuber surface structures were inspected and images taken using reflected light microscopy. The specimen was further dehydrated through an ethanol series, then Critical Point dried (Leica EM300 CPD), gold sputtered and imaged using the Phenom XL Benchtop Scanning Electron Microscope.

To compare the morphology of *Genoplesium baueri* tubers with tubers of *Corunastylis*, plants of *Corunastylis fimbriata* were excavated from private land in nearby Arcadia, NSW (with the owner’s permission). Characteristics of the *Corunastylis fimbriata* tuber including colour, nature of the tuber surface, presence of a fibrous tunic and arrangement of tubers on the plant (Jones & Clements 1989) were recorded and compared with that of *Genoplesium baueri* tubers.

During the 2019 growing season, an intact pollinarian was collected from the anther of a flower of *Genoplesium baueri*, photographed (Canon S120, microscope at X4 magnification) and its components identified (Dressler 1981). The pollinia of a pollinarian collected in the 2016 growing season were teased apart with probes and stained with Calberla’s Fluid (Ogden et al. 1974). The appearance of the pollen and other components and the size relative to a scale bar were captured in micrographs (Leica M125).
To determine the dispersal vectors of the pollen, insects carrying pollinaria were collected opportunistically from the flowers of *Genoplesium baueri* plants at KWG and from *Corunastylis fimbriata* plants at Arcadia, where no other flowering orchid species were present. The insect specimens were frozen before being identified to genus level and photographed (Canon S120 and Leica).

**Results and Discussion**

*Emergent plant structure, flowering and phenology*

*Genoplesium baueri* produces a tubular leaf, which surrounds the inflorescence scape and is terminated by a very short free lamina at a variable distance below the lowest flower of the inflorescence (Fig. 1). The convolutely furled leaf lamina emerges from within the below-ground, outer sheathing bract and grows upwards through a mostly shallow depth of soil and leaf litter to reach the surface. The outer sheathing bract has an inclined upper edge similar to the main leaf and was rarely visible above-ground level (Fig. 1). It was described by Nicholls (1969) as a “scarious sheath,” and, although observed on all tubers, was depicted but not mentioned in the literature (Fitzgerald 1886; Rupp 1943; Riley & Banks 2002; Jones 2006).

Flower buds are evident as bulges contained within the leaf lamina. As the plants develop, an inflorescence bearing one to fifteen (median 4, n=1,867), sessile, upwardly pointing buds emerges. Each flower remains non-resupinate and curved ovary are yellow-red to green. The lanceolate labellum is primarily dark red (Fig. 1).

The timing of developmental stages varies but in general leaves and flower buds appear in mid-Feb, flowering is well underway by mid-March, pollination and fertilisation are evident in early April and fruit dehiscence in early May (Fig. 2). Once the inflorescence has withered, the above-ground component of the plant dies back, but we were unable to determine if each individual plant had died or remained viable as a dormant, underground tuber. At KCNP, *Corunastylis ruppii, Corunastylis fimbriata* and *Corunastylis filiformis* were in flower with *Genoplesium baueri* in most years (*pers. obs.*). However, no hybrids between these orchid species were observed.

**Fruit**

After pollination and fertilisation, the decurved inferior ovary becomes swollen, straightened and changes colour from green to a yellow or brown (Fig. 1) while the sepals remain succulent. The mean length of six green capsules measured was 5.02 mm (s.d.=0.30 mm) while the mean diameter was 2.16 mm (s.d.=0.30 mm). The ripe fruit split along six sutures forming three narrow and three slightly wider valves (Fig. 1), allowing the fine orchid seed to fall to the ground or to be blown, washed or brushed out of the dry, paper-thin capsule. Unfertilised ovaries remained decurved and slim and were readily distinguished from the straightened, fertilised ovaries and swollen, developed fruit, as had been observed in the Tuncurry Midge Orchid by Bower et al. (2015). A distinguishing feature of *Genoplesium baueri* is that the sepals remain succulent right up until the seed release stage.

**Seeds**

The papery testa of each seed was estimated microscopically to be approximately 0.2-0.4 mm long by 0.1-0.2 mm wide. The embryo was estimated to be about 0.1 mm long (Fig. 1e). The embryos of *Genoplesium baueri* seeds were similar in size to the embryos of *Corunastylis filiformis* but the surrounding testas were slightly larger in *Genoplesium baueri*, which would provide greater buoyancy and dispersal potential. The number of seeds per capsule of *Genoplesium baueri* is large but the number of seeds and the proportion of viable seeds has not been estimated in this study. The minute size of germinating Australian terrestrial orchid seed (Stoutamire 1963; Dressler 1981; Batty et al. 2001) is likely to preclude their incidental observation *in situ* earlier than 4 to 5 months after germination. Several small protocorms/ tubers (3-9 mm long) were observed in May in soil associated with seed released from a mature plant in March the previous year, giving a possible age of 14 months (Grimm 2019).

![Image](Link to Image)
Tubers

The protocorms develop into ovoid tubers which have a smooth, tubular collar region at the apex where the above-ground shoot emerges from the tuber. Filamentous roots are not evident. The remaining surface of the tuber has scattered, small, raised clusters of papillae (Fig. 1, 4). Tubers less than about 7 mm in height produce few flowers whereas inflorescences of five or more flowers developed from larger, irregularly-shaped tubers (Grimm 2019). In addition, a scar on one side of the base of the tuber, mirrored by a polyp outgrowth on the opposite side, observed in most large, mature tubers, suggests lateral growth of a rhizome-like structure (Fig. 1a) capable of producing new inflorescences sequentially over time. In contrast, plants of sister taxa Corunastylis fimbriata bore smooth, vertically sequential spherical tubers, which lacked papillae and had filamentous roots emanating from above the tuber (Grimm 2019).

*Genoplesium baueri* plants have been described as growing alone or in loose clumps (Jones 2006) and have not been reported to multiply by vegetative increase. However paired inflorescences are common, and tight clumps of plants (Fig. 3) containing upwards of five plants growing within an area of about 10 cm square, although less common, occurred at both sites, indicating that vegetative reproduction may occur. Several natural processes could be invoked to explain such closely spaced plants. Firstly, instead of being dispersed, seeds may fall to the base of the mature plant and germinate (Jersáková & Malinová 2007). Secondly, if seeds are transported by dispersal vector, they may have accumulated at the base of plants such as Cyathochaeta diandra and Lomandra spp., where they were deposited (Arditti & Ghani 2000, Grimm 2019). Finally, multiple new plants may have resulted by vegetative reproduction (Pate & Dixon 1982; Grimm 2019) through budding polyps or beads on an existing mature tuber (Fig. 1a, 4a). Observation of active growth in tubers of plants which were releasing seed strongly suggests that this species is not monocarpic (Grimm 2019) and suggests that the formation of a polyp by lateral growth of a rhizome-like structure results in the generation of a new tuber. The narrow connection between the old and new tuber (Fig. 1a) is lost but a scar of the connection remains on the new tuber (Nicholls 1948, fig. 172; Grimm 2019). To ascertain the relationship between tubers and plant emergence across years, genetic investigations would be required to identify ramets and genets, which could then be used to determine the spatial distribution of individual plants.

Microscopy revealed that the clustered papillae on tubers were translucent and that the tips were coloured brown (Fig. 3a, b). Papillae on more recently developed parts of the tuber were less darkly coloured (Fig. 1a). Further examination by scanning electron microscopy (Fig. 4c, d) supported the proposal that the papillae contained pelotons (hyphal coils) of mycorrhizae.

**Fig. 2:** Phenology of *Genoplesium baueri* showing median times of commencement of each stage: (a) buds (mid-Feb), (b) flowering (mid-March), (c) pollination (early April) and (d) fruit dehiscence (early May) against mean monthly rainfall and monthly mean maximum temperature of the period (2009-19). Plants are deciduous with no above ground presence July to December. Climate data were obtained from the Bureau of Meteorology All Weather Station, Terrey Hills (Australian Bureau of Meteorology; No. 66059, Lat: 33.69° S, Lon: 151.23° E).

**Fig. 3:** Naturally occurring (a) tight clump of 14 *Genoplesium baueri* plants (at KWG) and (b) 11 variously sized tubers in a second clump of closely-growing plants revealed from beneath a decayed branch (at KWG). The tubers were too closely packed to track individual plants between years.

**Fig. 4:** *Genoplesium baueri* tuber micrograph: (a) lateral vertical section with small, spheroidal beads and clusters of papillae on outer surface and scar of weakly evident vascular trace intersecting side of tuber (scale bar 1 mm), (b) clusters of darkly coloured papillae on surface of tuber (scale bar 200 µm). Scanning electron
micrograph (images S. Lindsay): (c) papillae on surface of tuber (scale bar 100 µm), (d) papillae showing possible pelotons (hyphal coils) of mycorrhizae exposed (scale bar 10 µm).

Pollen

In the pollinarium of *Genoplesium baueri*, tetrad pollen grains were observed to be interconnected with other tetrads to form wedge-shaped units within the sectile pollinia (Fig. 5). The genus *Genoplesium* was described as having four pollinia in two caudicle-bearing pairs (Jones 1993). However, the mature pollinarium of *Genoplesium baueri* appears to have only two pollinia and a hamulus, comprising a single, broad translucent stipe attached to the viscidium (Rasmussen 1982; Kurzweil et al. 2005).

Although *Genoplesium baueri* has been described as self-pollinating (Jones 2006), myophily has been observed. Tiny flies (family Chloropidae, genus *Conioscinella*) carrying one to three pollinaria of *Genoplesium baueri*, were observed and captured on *Genoplesium baueri* plants. The pollinaria were attached by the viscidium in a post-occipital position to the back of the head of the fly (Fig. 5d, e). This contrasts with the position of attachment of the pollinaria of *Corunastylis fimbriata*, which attach on the dorsal surface (Fig. 5f) of the thorax of chloropid flies (Bernhardt et al. 2017; Ren et al. 2020).

Lifecycle

In the schematic diagram showing the proposed life stage transitions (Fig. 6) that we inferred from our observations from 2009-2020, we have indicated that flowering of an individual is unlikely to occur in the first year of development of the protocorm from seed, and an inflorescence is unlikely to occur in every consecutive year after the first flowering of the tuber. We have no evidence of the longevity of individual tubers and how frequently tubers flower in subsequent years, because plants are deciduous and individual plants cannot be reliably tracked between years. Observation of active growth in the tubers of plants, which are concurrently releasing seed, strongly suggests that this species is not monocarpic. In subsequent years the tuber appears to increase in volume and expand laterally. Vegetative propagation has not been included as a possible mechanism in the diagram; a plant produced in this manner would be more likely to reach maturity and to flower in less time than taken by a plant growing from seed, as is common in cultivated Orchidoid orchids. It is important that the likelihood of vegetative increase and clonality in *Genoplesium baueri* be further investigated (Bustam et al. 2014; 2016) as species-specific understanding is important in the conservation of rare species (Brundrett 2007).

Fig. 5: Pollinarium of *Genoplesium baueri* showing (a) stipe and viscidium and (b) pollinia. (c) Tetrad pollen grains form wedge-shaped units in pollinia of *Genoplesium baueri* (in Calberla’s Fluid stain, x400, scale bar 50 µm, image M. Renner). Flies in family Chloropidae, genus *Conioscinella*, carrying pollinaria of (d, e) *Genoplesium baueri* and (f) *Corunastylis fimbriata* (images J. Martin). Scale bars (a, b, c) 500 µm, (c) 50 µm, (d) 1 mm, (f) 200 µm.

Fig. 6: Schematic diagram of the proposed life-stage transitions of *Genoplesium baueri* as the plant proceeds from one stage of the sexually reproductive cycle to the next. In addition, there may be vegetative reproduction whereby tubers develop and separate to form new ramets. Arrows indicate possible movement between stages; dotted arrows represent movements that are less likely to occur. Arrows indicating movement to outside the oval represent death of the unit. Grey indicates units and processes that are proposed but cannot be observed directly because the unit exists underground.

Conclusion

Frequent visits to the sites within the northern distribution of *Genoplesium baueri*, and timely observations have provided some much-needed insight into the biology and lifecycle of this rare orchid species. Records of tuber development, observed active growth in tubers at the onset of the ‘dormant’, non-flowering period and the possibility of vegetative increase have been brought together to provide
one of several explanations for the formation of tight clumps of flowering plants in these populations. Chloropid flies transporting *Genoplesium baueri* pollinaria were observed, bringing into doubt the likelihood of this species being an obligate self-pollinator. These empirical observations will greatly contribute to informing management decisions, and ensure the conservation and persistence of this rare orchid species into the future.

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References


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