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Searching for Pretty Beards: A descriptive ecological model and targeted surveys for the rare *Calochilus pulchellus* (Orchidaceae)

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Abstract: *Calochilus pulchellus* (family Orchidaceae) (Pretty Beard Orchid) is a listed Endangered species with a highly restricted distribution in the Shoalhaven local government area. The species was only known from a small number of populations when described in 2006. Since then very few new populations of the species have been recorded and some previously known populations have not been observed for decades.

This study summarised known information regarding the distribution and habitat preferences of *Calochilus pulchellus,* with the aim of identifying potential habitat where unknown populations of the species may be detected by targeted surveys. Areas of potential habitat for the species were identified based upon vegetation communities in which the species had previously been recorded. A subset of the initially large area of identified potential habitat was selected for targeted surveys based on the recorded occurrence of sympatric threatened orchid species.

The targeted surveys resulted in an approximately three-fold increase in the previously known population size of *Calochilus pulchellus,* a small range extension for the species and a doubling of the extent of occurrence. Newly discovered individuals and populations of the species have increased the understanding of habitat for the species, with a greater proportion of the total known population of the species now known to occur within woodland and forest vegetation communities rather than heathlands. Despite the additional individuals of the species detected as part of this study, the species is still only known from a very low number of known individuals and a small number of populations and has a very high risk of extinction. Consequently, identifying and protecting additional populations of *Calochilus pulchellus* should remain a priority for the conservation of the species.

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Introduction

Population size, in combination with geographic range and decline, form the main part of the global standard for assessing extinction risk in the International Union for the Conservation of Nature (IUCN) Red List criteria (IUCN 2012). Population size also forms part of the formal assessment process for determining the risk of extinction of an individual species under Australian legislation (Commonwealth *Environment Protection and Biodiversity Conservation Act 1999; NSW Biodiversity Conservation Act 2016*).

In addition to small population size, specialisation with a partner organism is another factor which has been identified as potentially increasing the likelihood of extinction (Reiter et al. 2018). All orchid species have a specialised, symbiotic relationship with mycorrhizal fungi which they are dependent upon for germination in the wild (Warcup 1971; Bates & Weber 1990). Orchid species will inevitably decline if their symbiotic mycorrhizal fungi decline. Many species also have a specialised, symbiotic relationship with insect pollinators, including species of *Calochilus*.

Calochilus pulchellus (family Orchidaceae) (Pretty Beard Orchid), is a rare terrestrial orchid with known populations restricted to the Sydney Basin Bioregion and the Shoalhaven Local Government Area (Figure 1). The earliest collection of the species is the Type specimen from Morton National Park collected in November 2004, although the species was first recorded in the 1990s within the Jervis Bay area (Alan Stephenson pers. comm. 2018). When the species was described, it was identified as being known from only three populations: Morton National Park; Booderee National Park, and on private land in the suburb of Vincentia (Jones 2006a). By 2011, the total number of known sites for the species had increased to five locations (Stephenson 2011).

In 2011 the known population size of *Calochilus pulchellus* was 32 individuals (Stephenson 2011). Monitoring of the species between 2015 to 2018 (as part of the NSW Government's Saving Our Species program) identified up to 14 individuals, with between four and eight individuals observed in any specific year. The species has been identified as facing a very high risk of extinction due to the very low number of known individuals, the small number of populations and its highly restricted geographic distribution (NSW Scientific Committee 2008). *Calochilus pulchellus* is listed as Endangered under Part 2 of Schedule 1 of the NSW *Biodiversity Conservation Act 2016*.

Isolation of mycorrhizal fungi within roots of *Calochilus* species have identified the fungi as belonging to the Tulasnellaceae (Warcup 1981), a widespread orchid mycorrhizal fungus in both temperate and tropical regions (Reiter et al. 2018). Additional unknown fungi have also been identified as being associated with *Calochilus* spp. (Warcup 1981). There are currently no established protocols for the germination of *Calochilus* seeds and propagation of the species is recognised as being notoriously difficult (Backhouse & Jeanes 1995). This difficulty has been attributed to a complex and ongoing fungal dependence throughout their life cycle (Gavin Phillips pers. comm 2018; P. Weston pers. comm. 2018). In late 2018 a research project commenced with the Australian Botanic Garden at Mount Annan, seeking to develop techniques to effectively germinate and raise *Calochilus pulchellus* seedlings for a potential future translocation trial.

Calochilus pulchellus is at a very high risk of extinction due to the very low number of known individuals, specialisation with a partner organism (mycorrhizal fungi and pollinating wasps) and the current lack of successful nursery germination of seeds for the genus. An important element of efforts to conserve and protect *Calochilus pulchellus* is the management of threats at existing populations, which is dependent upon identification of populations of the species.

This study aimed to identify and survey potential *Calochilus pulchellus* habitat within the Shoalhaven Local Government Area (Figure 1) to detect previously unrecorded locations and populations of the species, so that these can be appropriately managed in the future, to increase the likelihood of securing the species' long-term conservation.

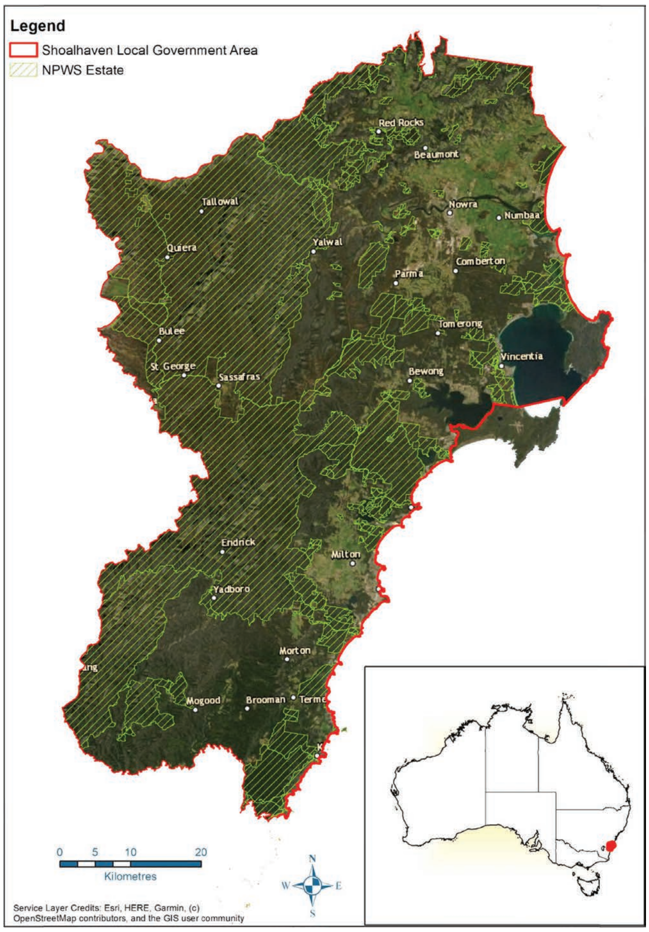

Figure 1: The Shoalhaven Local Government Area

Methods

Records of *Calochilus pulchellus* were collated from the National Herbarium of New South Wales, BioNet Atlas (the NSW Department of Planning, Industry and Environment) and the Australasian Virtual Herbarium (AVH). All records were manually reviewed to determine the record location, precision, and accuracy. Where the accuracy or precision of records was poor (limited to a single AVH record), collector's notes were reviewed, and location information was updated accordingly.

The vegetation communities in which *Calochilus pulchellus* had been previously recorded were determined using a Geographic Information System (GIS; ArcMap), from the intersect of records of the species and vegetation mapping of Tozer et al. (2010). Other environmental datasets potentially useful to developing a habitat model for *Calochilus pulchellus*, including climatic data, soil landscape mapping and vegetation height (LiDAR), were reviewed. However, the resolution of these datasets was either too coarse for the small area of occupancy of *Calochilus pulchellus*, or the datasets did not cover its entire range. Identification of potential *Calochilus pulchellus* habitat was based on the vegetation mapping of Tozer et al. (2010), which modelled the spatial distributions of map units across south-east NSW using some 60 environmental and spatial variables including soil landscape, vegetation structure, aspect indices, solar radiation, topographic roughness, topographic position and distance to streams (Tozer et al 2010).

The identification of the sympatric threatened orchid species *Prasophyllum affine* and *Cryptostylis hunteriana* at one of the known locations of *Calochilus pulchellus* was based upon our knowledge of the suite of flora species occurring at this location. Although the flowering period of the three species generally does not overlap at this location, we have observed them occurring within approximately 125 m of one another. To protect extant populations from poaching, specific locations are not included within this paper, following the Sensitive Species Data Policy in New South Wales (Andrews 2009).

Survey sites for *Calochilus pulchellus* were identified using GIS software based upon an intersect of mapped locations of those vegetation communities in which the species had previously been recorded (Tozer et. al. 2010); areas within a 530 m buffer around records of *Prasophyllum affine* and *Cryptostylis hunteriana*; and within National Park estate. The 530 m buffer was based upon the method for mapping threatened species for inclusion in the Biodiversity Values and the Native Vegetation Regulatory Maps. Survey sites were identified and prioritised within National Park estate due to the security of tenure and associated increase in feasibility/likelihood of successful long-term conservation actions for any newly identified populations.

Targeted surveys for *Calochilus pulchellus* were undertaken methodically on 4, 5, 6 and 27 November 2019 and opportunistically on other occasions throughout the flowering period. Surveys were conducted in accordance with the 'NSW Guide to Surveying Threatened Plants' (OEH 2016). Targeted surveys involved surveys along parallel transects in areas of potential habitat. Transect separation ranged from approximately 2.5 m – 5 m. In addition to parallel transects, random meanders through portions of survey sites were also undertaken to maximise the area covered by the surveys. Total survey effort included 76 km of survey tracks over approximately 56 person hours. The total survey area is estimated at 28.4 ha based upon the length of survey tracks and width of parallel transects (between 2.5 m and 5 m separation).

Various aspects of the lifecycle of *Calochilus pulchellus* have been linked to rainfall, including leaf emergence and the timing and abundance of flowering (Stephenson pers. comm. 2018). Rainfall for the six months preceding the survey period was slightly below average with a total of almost 550 mm of rainfall recorded from May to October 2019 at the Jervis Bay Airfield AWS meteorological station. The average rainfall over this six month period, as recorded at the Jervis Bay (Point Perpendicular AWS) meteorological station from 2001 to 2019, was 577 mm. The rainfall recorded for each of the six months preceding the survey period was variable with wet conditions in June and comparatively dry conditions in May, July and August (Figure 2). The timing of rainfall events for the six months preceding the survey period were generally regular with few lengthy periods without any rainfall (Figure 3). The longest period without any recorded rainfall occurred from the 14 October to 3 November, which was the 20 days immediately preceding the targeted surveys.

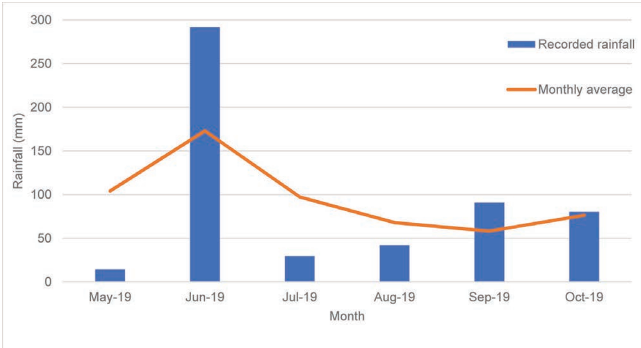

Figure 2: Monthly rainfall for the six months preceding the surveys (source: Jervis Bay Airfield AWS) and average rainfall from 2001-2019 (source: Jervis Bay Point Perpendicular AWS)

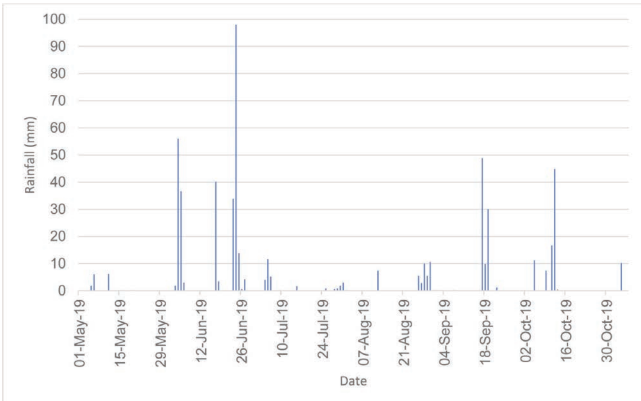

Figure 3: Daily rainfall for the six months preceding the surveys (Source: Jervis Bay Airfield AWS meteorological station)

Results

A total of 108 confirmed individuals of *Calochilus pulchellus* were recorded during targeted surveys in November 2019 (Figure 4). A further eight individuals were thought to be

Calochilus pulchellus, however flowers were either in bud or closed, such that a definitive identification to species level could not be made (hereafter referred to as 'probable' records). Two observations were also made of leaves of a *Calochilus* sp., but identification of these individuals could only extend to the level of genus. The 116 individuals of *Calochilus pulchellus* recorded in November 2019 (including both confirmed and probable records) includes 27 individuals identified as part of monitoring surveys at previously known locations (with 13 individuals newly identified in 2019).

Excluding the 27 individuals identified as part of monitoring at previously known locations, the remaining 89 individuals represent previously unknown locations for the species, excepting the single individual at Woollamia Nature Reserve where a single individual was observed 20 years ago (A. Stephenson pers. comm. 2019) although no individuals had been seen again until the surveys in Spring 2019.

The northernmost observations of *Calochilus pulchellus* from Jervis Bay National Park, in the vicinity of Callala Bay, represents a northerly extension of its known range by approximately 5 km. All other observations are located within the previously known range of the species.

Prior to the surveys, the Area of Occupancy (AOO) for *Calochilus pulchellus* was two 10 x 10 km grid cells and the Extent Of Occurrence (EOO) was approximately 100 km^2 (Bland et al. 2016). Based upon our additional confirmed 2019 observations, the AOO is four 10 x 10 km grid cells and the EOO is approximately 260 km^2.

The targeted surveys for *Calochilus pulchellus* were in areas where the mapped vegetation community (Tozer et al. 2010) matched the mapped vegetation communities at previously known populations of the species. Consequently, all additional records were located within vegetation communities in which the species had previously been recorded. However, field observations of vegetation communities identified that some confirmed habitat for *Calochilus pulchellus* previously mapped as 'Currambene-Batemans Lowlands Forest – p85' (Tozer et al 2010; equivalent to Plant Community Type [PCT] 1079: Red Bloodwood - Blackbutt - Spotted Gum shrubby open forest on coastal foothills, southern Sydney Basin Bioregion) was actually more akin to 'Shoalhaven Sandstone Forest – p148' (Tozer et al 2010; equivalent to PCT 1082: Red Bloodwood - Hard-leaved Scribbly Gum - Silvertop Ash heathy open forest on sandstone plateaus of the lower Shoalhaven Valley, Sydney Basin Bioregion). This represents a new vegetation community in which no previously documented records of *Calochilus pulchellus* have been made.

Figure 4: Newly discovered individual of Calochilus pulchellus within Jervis Bay National Park (Photo: B. Towle)

Table 1: The mapped extent of vegetation communities (Tozer et al. 2010) in which Calochilus pulchellus has been recorded

Vegetation community (after Tozer et al. 2010)	Area within Shoalhaven LGA (ha)	Area within Shoalhaven LGA and National Park estate (ha)
Budderoo-Morton Plateau Forest – p141 (PCT 1152)	2,982	2,908
Coastal Sand Swamp Forest – p45 (PCT 1231)	1,151	337
Coastal Sandstone Plateau Heath – p117 (PCT 882)	6,056	1,736
Currambene-Batemans Lowlands Forest – p85 (PCT 1079)	24,616	5,043
Floodplain Swamp Forest – p105 (PCT 1234)	1,451	375
Morton Mallee Heath – p122 (PCT 662)	33,081	29,864
Shoalhaven Sandstone Forest – p148 (PCT 1082)	39,440	24,569

Discussion

Area and Extent of Occurrence

The newly discovered locations of *Calochilus pulchellus* in this study have more than doubled the EOO of the species (from approximately 100 km^2 to approximately 260 km^2). However, the AOO of the species remains low: four 10 x 10 km grid cells. This discrepancy between the EOO and AOO, as well as the mapped extent of vegetation communities in which the species has been recorded (Table 1), highlights the large areas of potential but unconfirmed habitat for the species within its known distribution. Based upon the available habitat and the results of these targeted surveys, there is a reasonable likelihood that additional targeted surveys for *Calochilus pulchellus* in areas of potential habitat, within the species known distribution, would locate additional populations of the species. The identification and protection of additional populations of *Calochilus pulchellus* should remain a priority for the conservation of the species, particularly while established protocols for the *ex-situ* germination of seed remain elusive. The identification of survey sites for the 2019 targeted surveys was based upon equivalent vegetation types and sympatric orchid species. This method successfully identified new populations and a similar method should be considered for site selection for any future targeted surveys.

Habitat

The habitat associated with *Calochilus pulchellus* has previously been described as predominately heathland vegetation (Jones 2006a, Jones 2006b, Stephenson 2011). Specifically, the known habitat has variously been described as: dense low heathland with scattered emergent *Eucalyptus* and *Banksia*; wet heath in well drained to wet sand over sandstone (Jones 2006a, Jones 2006b, Stephenson 2011); and 'Scribbly Gum *Casuarina* Forest' in dry well drained sandy soil (a single individual observed in 2014). However, additional populations and records in this study have increased the number of *Calochilus pulchellus* individuals observed within woodland and forest vegetation communities such that the greater proportion of the total known population of the species occur within woodlands and forests rather than heathlands (Figure 5).

Figure 5: Confirmed habitat for *Calochilus pulchellus*, corresponding to the Shoalhaven Sandstone Forest of Tozer et al. (2010; Photo: B. Towle.)

Microhabitat

Specific microhabitats in which *Calochilus pulchellus* was recorded during this study included areas where there was increased solar exposure of the groundlayer and sites with understorey species often associated with swamps, swamp margins and increased moisture availability. Additionally, *Calochilus pulchellus* was frequently observed growing at, or in proximity to, the bases of large trees (predominately *Eucalyptus sclerophylla* and *Corymbia gummifera*) in deep leaf litter, which may represent conditions which favour the mycorrhizal fungi on which the species is dependant. Given the bias of the surveys towards the identification of reproductive individuals of *Calochilus pulchellus*, it is unknown whether the species is more common in the microhabitats described above, or if these conditions are more conducive to flowering than individuals occurring in more shaded areas. Irrespective of whether increased exposure of the understorey increases flowering of the species, or whether it is a habitat requirement for the occurrence of the species, any future targeted surveys should include areas with this microhabitat.

Flowering

The first open flower of *Calochilus pulchellus* in 2019 was observed on the 18 October 2019, with peak flowering estimated to have occurred during the first week of November 2019, similar to previous 2015 – 2018 seasons where peak flowering occurring on the last week of October or early November (unpublished Saving Our Species monitoring data). The generally similar flowering time over the last four years indicates that while the timing of the emergence of leaves from tubers may be triggered by rainfall events, or more likely the interaction between rainfall events and declining temperatures, the timing of flowering is likely to be influenced by other factors which may include day-length and temperature cues. Due to the large discrepancies between the size of the identified sub-populations, no meaningful comparisons between the timing of peak flowering at different sub-populations is possible. However, results of this study suggest that peak flowering may occur earlier in the northern *Calochilus pulchellus* population (Callala Bay) than more southerly populations, a phenomenon which has been observed for the sympatric orchid species *Prasophyllum affine*. Further monitoring would be required to confirm this discrepancy between peak flowering times.

Jones (2006b) reports that flowering of *Calochilus pulchellus* is enhanced in the seasons following fire (at the time of its formal description two populations were in areas burnt the previous season). Increased flowering post-fire is attributed to increased solar exposure, due to reduced competition from other vegetation and increased post-fire soil nutrients (M. Clements pers. comm. 2018). Monitoring following an ecological burn at the Type location in May 2017 did not record any flowering in 2017 or 2018 seasons, but seven individuals in 2019. This suggests that if fire does enhance flowering, the response can be delayed, or that the specifics of that fire event, including timing, temperature and time between burns, and post-burn rainfall may determine whether flowering is enhanced following fire. Much of the extent of occurrence of *Calochilus pulchellus* was impacted by high intensity fire activity in the summer of 2019/20, although no known populations were burnt. Further surveys for the species within areas which were impacted by fire activity may provide additional insights into the impact of fire upon flowering of the species.

Longevity

Across the *Calochilus* genus, it has been reported that few plants survive the flowering effort more than once or twice, and that flowering may often represent the climax of the life-cycle of an individual (NSW Scientific Committee 2008; M. Clements pers. comm. 2018). As daughter tubers are not produced by *Calochilus pulchellus* (Jones 2006a), the species is reliant on recruitment from seed rather than vegetative persistence (NSW Scientific Committee 2008; M. Clements pers. comm. 2018). The observation of *Calochilus pulchellus* at the same location some 20 years after its initial discovery, either as a long-lived individual with multiple flowering events or as offspring from a parent plant, indicates that recorded locations of the species can support populations over long time periods. While not conclusive, the continued observation of flowering individuals of *Calochilus pulchellus* within one of the monitored populations from 2015 to 2019 (including flowering stems present within near identical locations based upon triangulation from multiple nearby trees), suggests the species is capable of flowering over multiple seasons and some vegetative persistence occurs post-flowering. However, it is not possible to discount the persistence of flowering individuals within this population based upon recruitment from the seedbank. Irrespective of whether their continued presence is based upon a persistent tuber capable of repeat flowering or recruitment from the seedbank, as the species has been recorded at individual locations over long time periods (up to 20 years) appropriate management of these locations is important for the survival of the species.

Conclusion

Surveys undertaken as part of this study have resulted in an approximately three-fold increase in the previously known population size of *Calochilus pulchellus* and a small range extension for the species. Nonetheless, as the species is still only known from a very low number of known individuals and a small number of populations, it still has a very high risk of extinction. Identifying and protecting additional populations of *Calochilus pulchellus* should remain a priority for the conservation of the species, particularly while established protocols for the germination of seed remain elusive.

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