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Pollinator sharing by the sexually deceptive Greencomb Spider Orchids, *Caladenia parva*, *C. phaeoclavia* and *C. villosissima* (Orchidaceae: Caladeniinae): taxonomic considerations

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Abstract

Sexually deceptive orchids are pollinated when male insects perform mating behaviours on the female-mimicking labellum. Such orchids are characterised by extreme pollinator specificity, having only one, or occasionally a few, closely related insect species as pollen vectors. Sharing of a sexually deceived pollinator species between two or more orchid species is rare among Australian orchids in the Drakaeinae and Caladeniinae pollinated by male Thynnid wasps. In this paper putative pollinator sharing within a complex of three morphologically similar species of Greencomb Spider Orchids, *Caladenia parva* G.W. Carr, *C. phaeoclavia* D.L. Jones and *C. villosissima* G.W. Carr, was investigated using pollinator baiting and choice testing in the field. The three orchid taxa are shown to share the same thynnid wasp pollen vector, *Lophocheilus anilitatus* (Smith) over the entire geographic range of the complex in New South Wales and Victoria. Pollinator baiting and choice testing at 42 locations revealed no evidence for the existence of cryptic pollinator species in *L. anilitatus*. The close morphological similarity of the three orchids, their sharing of the same pollinator and lack of evidence of a reproductive isolating mechanism, suggest they belong to the same biological species, *C. parva*. However, phylogenetic analysis is desirable to confirm the monophyly of the three morphospecies and the possible existence of polyploidy in *C. villosissima* merits investigation.

Introduction

Pollination by sexual deception of male insects has evolved independently in multiple lineages of the Orchidaceae principally on two continents, Europe and Australia, and involves hundreds of species (Gaskett 2010). Pollen transfer occurs in sexually deceptive orchids when female mimicking labellum structures stimulate mating behaviours in deceived males (Vereecken and Francisco 2014, Bower 2014). Sexually deceived male insects are attracted to flowers by odours (allomones) mimicking species-specific sex pheromones normally emitted by their females (Schiestl *et al.* 1999, 2000, 2003, Ayasse *et al.* 2002, Franke *et al.* 2009, Peakall *et al.* 2010, Ayasse *et al.* 2011). Sexually deceived male pollinators respond rapidly by flying upwind to picked flowers placed in the field, a procedure known as 'baiting' (Stoutamire 1975, Peakall 1990, Bower 1996).

Most sexually deceptive orchids are thought to be monophilous, attracting a single pollen vector species, or rarely, several closely related species. Such extreme pollinator specificity has long been considered a key characteristic of sexual deception (Stoutamire 1975, Bergstrom 1978). Experimental evidence for pollinator specificity in sexually

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deceptive orchids requires the exposure of arrays of flowers of different species to pollinator populations in the field (Paulus and Gack 1990, Bower 1996). Specificity is demonstrated by consistent assortative responses to each orchid species by pollinators. Such techniques have demonstrated pollinator specificity among sexually deceptive species of *Chiloglottis* pollinated by Thynnid wasps of the genus *Neozeleboria* in south-eastern Australia (Bower 1996, Bower and Brown 1997, Bower 2006, Bower and Brown 2009, Peakall *et al.* 2010, Whitehead and Peakall 2014). Similarly, Phillips *et al.* (2014) revealed extreme pollinator specificity in the Thynnid pollinated genus *Drakaea* across its distribution in the south-west of Western Australia.

An important consequence of monophily is that, with only one exception, sympatric *Chiloglottis* and *Drakaea* species do not share pollinators, that is, each orchid species has a unique single pollinator within its natural distribution (Bower 1996, Bower and Brown 2009, Whitehead and Peakall 2014, Phillips *et al.* 2014). The exception is the mutual attraction of each other's pollinators by *Chiloglottis valida* and *C. trapeziformis*, where their usually separate altitudinal ranges and habitats overlap (Peakall *et al.* 1997, 2002). Accordingly, extreme pollinator specificity usually confers complete reproductive isolation between sympatric co-flowering sexually deceptive species (Xu *et al*. 2011, Whitehead and Peakall 2014). In addition, strict monophily allows the detection of cryptic pollinator-specific orchid biospecies and cryptic pollinator species through controlled pollinator choice testing (Bower 2006, Bower and Brown 2009).

By contrast to the lack of pollinator sharing in *Chiloglottis* and *Drakaea* revealed by field choice testing, published pollinator lists suggest pollinator sharing may occur in some groups of sexually deceptive, Thynnid-pollinated *Caladenia* species in eastern Australia (Phillips *et al.* 2009, Gaskett 2010). While most sexually deceptive *Caladenia* species attract unique pollinators, nine species in the *Caladenia reticulata* complex are pollinated by *Phymatothynnus* near *nitidus* 1, three species of Greencomb Spider Orchids are pollinated by *Lophocheilus anilitatus* and *Caladenia tesselata* shares *P*. near *nitidus* 1 with the *C. reticulata* group (Phillips *et al*. 2009, Gaskett 2010). These data contrast markedly with results for *Chiloglottis* and *Drakaea*, and suggest that factors other than pollinator specificity may govern reproductive isolation in some *Caladenia* clades. Potential factors include post-zygotic incompatibility and different positioning of pollinaria on the body of the pollinator (Paulus and Gack 1990, Phillips *et al*. 2013, Vereecken and Francisco 2014). However, all Thynnid pollinators carry the pollinia of Drakaeinae and Caladeniinae nototribically on the centre thorax discounting a role for differential pollen placement in reproductive isolation in these groups (Stoutamire 1983, Phillips *et al*. 2009). Alternatively, it is possible that cryptic pollinator species may exist within *P*. near *nitidus* 1 and *Lophocheilus anilitatus*, such that pollinator sharing may not be occurring. A further possibility is that a mismatch exists between morphospecies and biospecies boundaries in the Greencomb Spider Orchids and the *C. reticulata* complex (Phillips *et al*. 2009), which would suggest the two orchid complexes may have been over-split taxonomically.

Swartz *et al.* (2014) examined these issues in the *C. reticulata* complex. Extensive pollinator baiting and field choice tests throughout the distribution of the complex in western Victoria showed that ten species shared the single pollen vector *P. nitidus* (=*P.* near *nitidus* 1 of Phillips *et al*. 2009) and that no cryptic species existed within the *P. nitidus*. Phylogenetic analyses of 31 *Caladenia* species, including 25 in the reticulata complex, showed limited species level resolution within the complex and no differences between species with different pollinators (Swartz *et al*. 2014). Population genetic analyses using six polymorphic microsatellite loci in the ten pollinator sharing species found only limited differentiation from three species in the complex employing different pollinators. No differentiation was revealed between allopatric species in the complex or species with confirmed one-to-one pollinator relationships. Patterns of low molecular phylogenetic diversity and high morphological similarity among related species are common in orchids in general (Phillips *et al*. 2012) and sexually deceptive orchids in particular, for example *Ophrys* (Soliva *et al*. 2001, Soliva and Widmer 2003), *Chiloglottis* (Mant *et al*. 2005) and *Caladenia* (Swartz *et al*. 2009). Overall, the sharing of the same vector species among ten reticulata complex species combined with evidence of very low genetic divergence suggested they belong to the same biological species and most likely represent an example of taxonomic over-splitting (Swartz *et al*. 2014).

This paper uses pollinator baiting and controlled field choice tests to examine putative pollinator sharing (Phillips *et al*. 2009, Gaskett 2010) across the geographical distributions of three closely similar, sexually deceptive species of Greencomb Spider Orchids; *Caladenia parva* G.W. Carr, *C. phaeoclavia* D.L. Jones and *C. villosissima* (G.W. Carr) Hopper & A.P.Br. in south-eastern Australia, hereafter referred to as the *C. parva* group. The aims of the study were to determine: (1) the pollinator assemblage for the *C. parva* group across its geographic range; (2) whether each species in the group has a unique pollinator; and (3) whether any cryptic orchid or pollinator species are present. The taxonomic implications of the results are discussed.

Caladenia parva, C. villosissima and *C. phaeoclavia* were formally described in 1991. *C. parva* is a small species with short habit, small flowers, strongly deflexed lateral sepals, relatively short labellum fringes and small column (Carr 1991). Backhouse and Jeanes (1995) gave the distribution as 'southern Victoria, west from

Wilsons Promontory, and extending inland to the Grampians' and qualified their interpretation of the species by the statement 'Possibly no more than a poorly developed form of a more widespread species'. Carr (1991) distinguished *C. villosissima* (as *Caladenia dilatata* var. *villosissima*) by its 'densely villous leaf and thick scape' and gave its distribution as 'an area bounded by Stawell, the eastern Grampians and Maryborough' in central western Victoria. *Caladenia phaeoclavia* was distinguished by its short habit, relatively small flowers, brown sepaline osmophores, basal calli with prominently clavate rounded heads, narrow column wings and narrow column basal glands (Jones 1991). The distribution was given as New South Wales, Australian Capital Territory and possibly eastern Victoria.

Fig. 1. Flowers of *Caladenia parva* (Dergholm, Vic.) (left), *C. phaeoclavia* (Mullion Creek, NSW) (middle) and *C. villosissima* (Langkoop, Vic.) (right).

The recorded distributions of *C. parva* and *C. phaeoclavia* are confused due to the similarity of the two species (Backhouse and Jeanes 1995). This can be seen by comparing distribution maps given by Backhouse and Jeanes (1995), the Australian National Herbarium Canberra (CANB) website (http://www.cpbr.gov.au/cpbr/herbarium/) and the Australia's Virtual Herbarium (AVH) website (http://www.rbg.vic.gov.au/cgi-bin/avhpublic/avh.cgi). All three sources agree that *C. parva* is confined mainly to coastal southern Victoria and South Australia west from South Gippsland to the Eyre Peninsula, with an extension inland to the Grampian Ranges in western Victoria. The distribution of *C. phaeoclavia* given by the Australian National Herbarium Canberra (CANB), as determined by M.A. Clements and D.L. Jones, and of Backhouse and Jeanes (1995) is the Great Dividing Range south from the Northern Tablelands of NSW and eastern Victoria with an extension to the Grampians. By contrast, the distribution of *C. phaeoclavia* given by AVH shows complete overlap between it and *C. parva* in south western Victoria and South Australia, such that *C. parva* is represented as wholly sympatric with *C. phaeoclavia* in those areas. The AVH map includes many specimens at the National Herbarium of Victoria and the State Herbarium of South Australia that have been identified as *C. phaeoclavia* and originated from within the area considered to support only *C. parva* by CANB and Backhouse and Jeanes (1995). Nevertheless, it is generally agreed that *C. parva* is absent from New South Wales, coastal Victoria east of South Gippsland and the Great Dividing Range and inland slopes in Victoria, except for the Grampian Ranges. The close morphological similarity between *C. phaeoclavia* and *C. parva* is well illustrated by the photographs in Backhouse and Jeanes (1995), Jeanes and Backhouse (2006) and Fig. 1.

For consistency, Fig. 2 shows the distributions of *C. parva* and *C. phaeoclavia* for specimens identified by M.A. Clements and D.L. Jones and housed at CANB. In accordance with CANB, all populations in this complex in NSW and the eastern ranges of Victoria are considered in this paper to represent *Caladenia phaeoclavia*, and those in coastal regions from west of South Gippsland to South Australia are considered to represent *C. parva*. It is recognised that both species may be present in the Grampian Ranges.

Methods

Study species

Caladenia parva, C. villosissima and *C. phaeoclavia* are perennial terrestrial herbs belonging to the Greencomb Spider Orchid complex, which currently comprises 24 species (Jones 2006). Each has a single erect hairy leaf at ground level and a solitary flower stem with dense erect hairs bearing one, or rarely two, terminal flowers in *C. parva* and *C. phaeoclavia*, or one to three flowers in *C. villosissima*. Plants flower in spring and die back in summer to the underground tuberoid, which is replaced annually. A new leaf is produced following soaking autumn rains. Greencomb Spider Orchids are distinguished by the greenish labellum whose upturned sides bear prominent long comb-like fringes, a deep maroon curled labellum apex and prominent tall maroon calli in four central rows from the near the apex to the base (Fig. 1). The three species grow in open forests, woodlands and heaths often with shallow or poor soils.

Pollinator specificity tests

Pollinator baiting (Stoutamire 1983, Peakall 1990) and pollinator choice experiments (Bower 1996) were used to determine the pollinator specificity of flowers from ten populations of *Caladenia phaeoclavia*, eight populations of *C. parva* and three populations of *C. villosissima* (Fig. 3, Table 1). In order to ensure that *Caladenia phaeoclavia* in the strict sense was being tested, samples were taken from the type population of *C. phaeoclavia* (Wambool Nature Reserve, Yetholme, NSW) and a population cited in the description (Mullion Creek (site 2)), both originally collected by the author. Similarly, samples of *Caladenia parva* and *C. villosissima* for baiting were collected from their type areas near Dergholm and Stawell in western Victoria, respectively (Fig. 4, Table 2).

Baiting was conducted through much of the known ranges of the three taxa in New South Wales and Victoria (Fig. 4) in October and November in 1992, 1995, 1996 and 2006 to 2009. Most baiting was conducted as single species tests (Bower 1996) in which a single set of 3 flowers sampled from the same orchid population and species was presented by itself at a location where pollinator responses were obtained. Four such samples were tested only in the local area of the populations from which they taken (Table 1). Flowers from 6 populations were tested at 2 to 4 dispersed localities within the same region, e.g. central NSW or southern Victoria (Table 1). Samples from ten populations were tested both in their local area and translocated long distances, up to 750 km, to other regions for testing (Table 1). A sample from one population, Batlow on the NSW Southern Tablelands, was tested only after translocation to Mullion Creek on the NSW Central Tablelands (Table 1). A total of 41.7 hours of single species testing was conducted. In addition, 14 sequential choice tests (see below) were conducted in which sets of 3 flowers from multiple populations and species were presented to the same pollinator population sequentially. Sequential choice testing totaled 5.7 hours of test time.

Insects visiting sexually deceptive flowers vary considerably among individuals in the strength of their motivation. At the two extremes, many may not be duped by the flowers and fly away after a brief approach, while others land and interact vigorously with the labellum. Accordingly, criteria are needed for determining whether an insect that briefly visits in flight is considered to be responding to stimuli from a set of flowers. The criteria used in this study are: (1) approaching flowers directly in flight to within about 10 cm; (2) circling flowers closely in flight; and/or (3) landing on or within 5 cm of the flowers. The preceding criteria are the minimum requirements for capture of a visitor for identification. Following the initial attraction, insects may exhibit an escalating sequence of behaviours depending on the level of stimulus provided by the flowers and the drive of the insect. This sequence of behaviours was documented for each visit and categorized as (1) only approached the flowers in flight before flying away; (2) landed on any part of the flower; (3) attempted to mate with the labellum or tepal glands; (4) removed pollinia from the anthers; (5) arrived carrying pollinia from local flowers; or (6) pollinated the stigma of a test flower. Observation of behaviours 4 to 6 by any individual indicates the insect species is a capable pollinator (Bower 1996). Insects meeting any of the above criteria were captured, identified by Dr. Graham Brown (Museum and Art Gallery of the Northern Territory) and lodged in the Museum. Specimens of most orchid populations have been submitted to the Australian National Herbarium, Canberra.

For pollinator baiting, a set of three flowers on 15 cm stems from the same population was placed in a vial of water mounted in a wood block on the ground in natural habitat. Two or more sets of flowers from different populations mounted 40 cm or more apart were monitored simultaneously in choice experiments. Sets of flower were oriented in a line perpendicular to the prevailing wind direction to minimise overlap of allomone plumes close to the vials. Flowers were exposed for a standard 3 min at each site since most insects within range of the allomone arrive within the first few minutes of an exposure (Peakall 1990). Each experiment comprised several to many exposures at multiple sites within a locality supporting a pollinator population, until up to 20 insects had responded in total. Flowers were moved to new sites 20 m or more apart to attract additional insects by sampling a new segment of the pollinator population.

To compare pollinator responses to *Caladenia* samples from different populations, 'sequential choice tests' (Bower 1996) and 'sequential single species tests' were used. A sequential choice test was used to establish whether a lack of responses to one or more sets of flowers in a choice test was due to the dominant attractiveness of other samples. A sequential choice test consisted of: (1) a 3 min exposure of sets of flowers with low or nil responses in a previous choice test, followed at the same site by (2) adding previously attractive flowers to the array for an additional 3 min as a control. Responses by insects in the first 3 min to previously unattractive flowers show the flowers have similar allomones, albeit quantitatively or qualitatively different, and may represent the same biological species as the more attractive samples. Flowers that remain unattractive, when there is a positive response to the controls, may have different allomones and a different pollinator, and may represent a different cryptic orchid biospecies.

Sequential single species tests were used at some locations instead of sequential choice tests because of very high activity by responding insects at those locations. When two sets of highly attractive flowers were exposed together in choice tests, responding insects often failed to choose between them, instead flying in rapid loops from one to the other before departing. When each set of flowers was exposed singly, insects were able to pinpoint a single source of attraction, enabling them to home in, land and exhibit psedocopulatory behaviour. Accordingly, sequential single species tests involve 3 min exposures of single sets of flowers from different orchid populations, one after the other, within the same insect population.

The approach of Bower and Brown (1997) was used to test for the possible existence of cryptic pollinator species. The method relies on the natural patchiness of insect populations in nature, such that if there are two or more cryptic insect taxa, one or other of them will be absent at locations where sister taxa are present. Two orchid taxa with different cryptic pollinators will each exclusively attract insects at some locations. Accordingly, sequential choice tests or sequential single species tests were conducted with samples of the three *Caladenia* species at as many pollinator locations as possible.

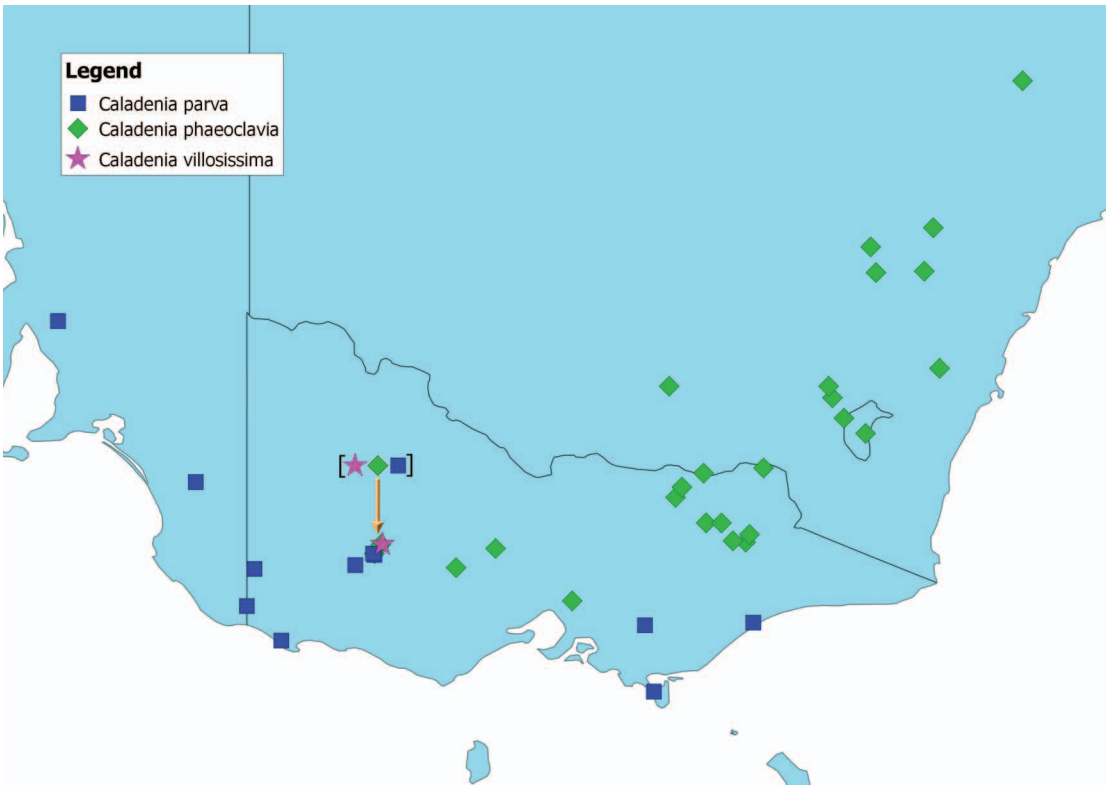

Fig. 2. Distributions of *Caladenia parva* and *C. phaeoclavia* based on specimens identified by M.A. Clements and D.L. Jones housed at CANB. The distribution of *C. villosissima* is based on two specimens at MEL.

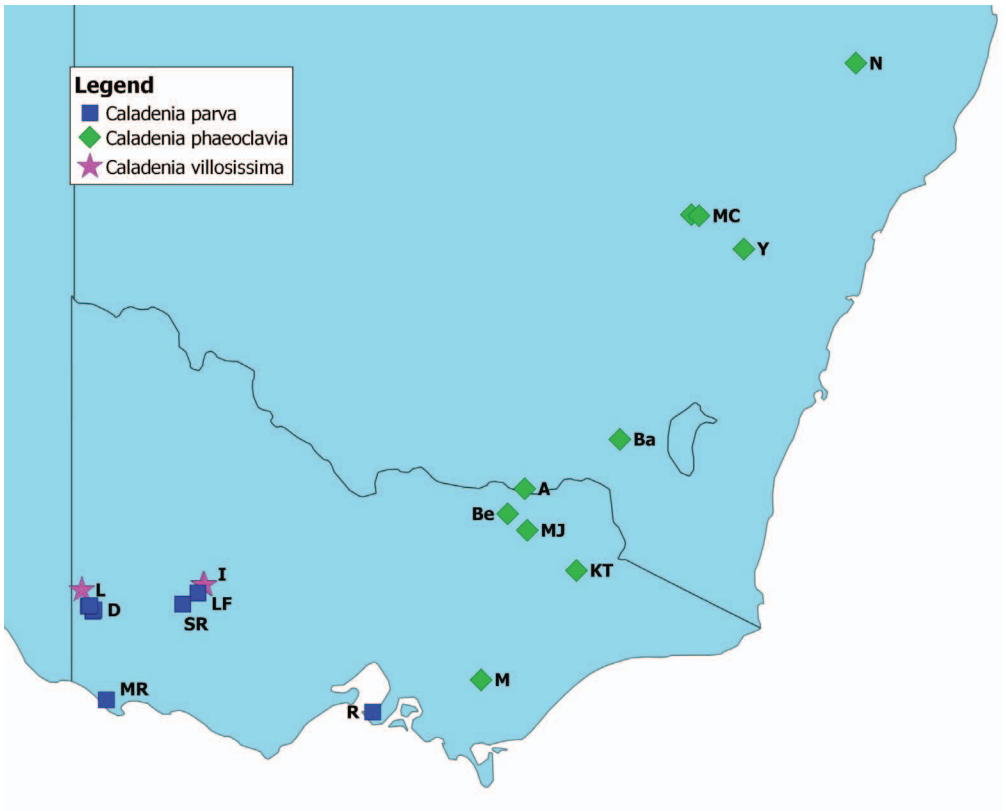

Fig. 3. Source locations of *Caladenia parva*, *C. phaeoclavia* and *C. villosissima* used for baiting. (Albury [A], Batlow[Ba], Beechworth [Be], Dergholm [D] – 4 locations, Illawarra [I] – 2 locations, Knocker Track [KT], Lake Fyans [LF], Langkoop [L], Moe [M], Mullion Creek [MC] – 2 locations, Mount Jack [MJ], Mount Richmond [MR], Serra Road [SR], Nundle [N], Rosebud [R], Yetholme [Y])

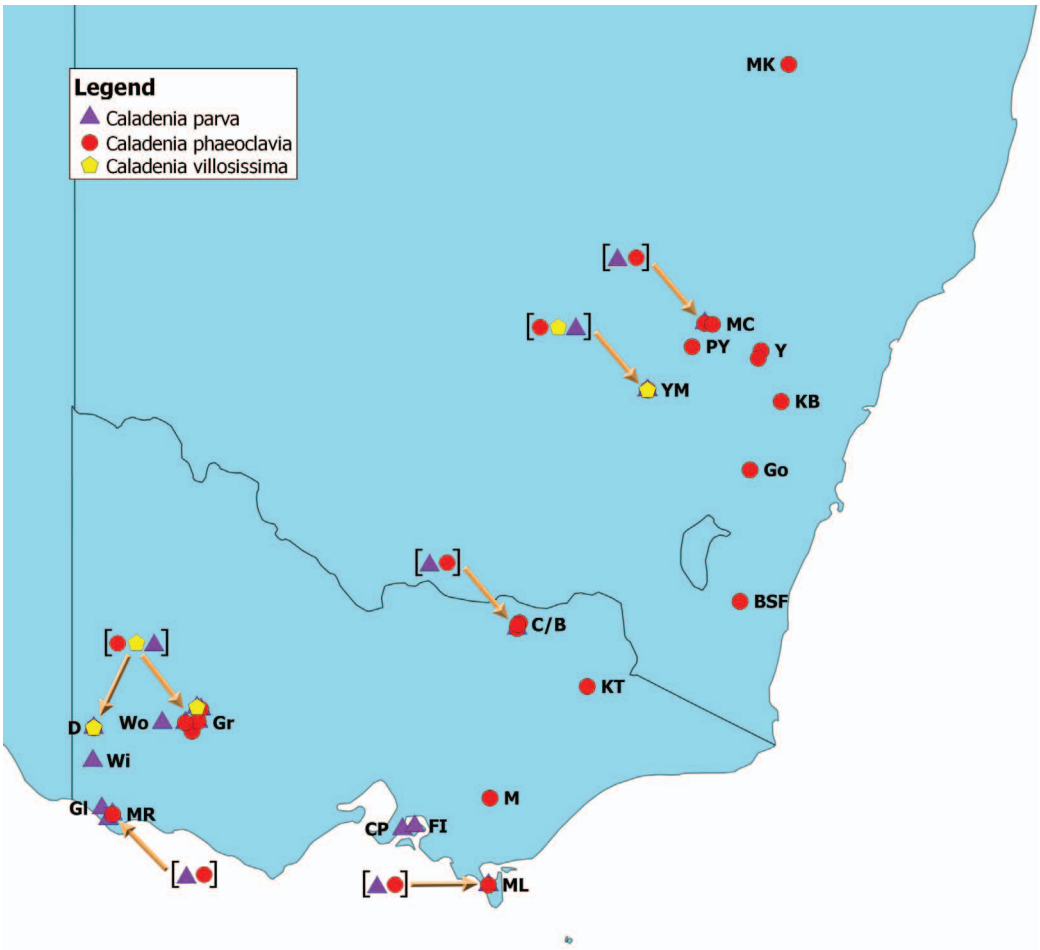

Fig. 4. Pollinator baiting locations at which responses by *Lophocheilus anilitatus* were obtained to *Caladenia parva, C. phaeoclavia* and/or *C. villosissima*. (Badja State Forest [BSF], Beechworth [B], Chiltern [C] (2 locations), Crib Point [CP], Dergholm [D], French Island [FI], Grampian Ranges [Gr] (5 locations including Lake Fyans and Devils Garden State Forest), Glenelg National Park [Gl], Goulburn [Go], Kanangra-Boyd National Park [KB], Knocker Track [KT], Mount Kaputar [MK], Millers Landing [ML], Moe [M], Mount Richmond [MR] (includes Cobobbonee State Forest), Mullion Creek [MC] (2 locations), Paling Yards [PY], Wilkin [Wi], Woohlpooer [Wo], Yambira Mountain [YM] (2 locations), Yetholme [Y] (2 locations),

Results

Pollinator observations

The thynnid wasp, *Lophocheilus anilitatus* (Smith) was the only insect visitor (Figs 5, 6) attracted to samples of *Caladenia phaeoclavia*, *C. parva* and *C. villosissima* throughout the area from Mt. Kaputar on the North West Slopes of NSW almost to the South Australian border in western Victoria (Fig. 4, Tables 1, 2). A total of 782 *L. anilitatus* was attracted in 47.4 hours of baiting time at 42 locations with populations of the pollinator (Tables 1 and 2). Of these, 402 *L. anilitatus* were attracted by *Caladenia phaeoclavia*, 323 by *C. parva* and 57 by *C. villosissima* (Table 1). *C. phaeoclavia* translocated from the NSW Central Tablelands attracted *L. anilitatus* at six locations in South Western Victoria, including Dergholm, the type area of *C. parva* (Tables 1 and 3). Conversely, *C. parva* samples from South Western Victoria attracted *L. anilitatus* in central western NSW (Tables 1, 3).

The behavioural interactions of *Lophocheilus anilitatus* with flowers showed it was capable of pollinating all three *Caladenia* taxa (Tables 1, 3, Figs 5, 6). Attempted mating by wasps on the labellum or lateral sepal clubs was recorded 125 times, representing 18.1% of the 690 interactions for which behaviour was recorded (Fig. 5, Table 3). Pseudocopulations by *L. anilitatus* were observed on flowers translocated up to 750 km between south western Victoria and eastern NSW and Victoria, and vice versa (Table 3). Removal of pollinia from the anthers of bait flowers was observed on 21 occasions (3.0% of interactions), including flowers translocated in both directions (Table 3). In all cases, pollinia were deposited in the same position on the centre of the wasp's dorsal thorax. Only two pollinations of flowers were observed, both vector-mediated self-pollinations in which highly motivated wasps removed pollinia from the anthers of a flower and subsequently transferred it to the stigma of the same flower.

Fig. 5. Male thynnid wasp *Lophochocheilus anilitatus* arching its abdomen and probing the labellum lamina of *Caladenia phaeoclavia* with its genitalia.

Fig. 6. Male *Lophocheilus anilitatus* pushing the anther covers of *Caladenia phaeoclavia* open with its thorax.

In some places, notably Mullion Creek (site 1), Chiltern (site 1) and Beechworth in the east, and Lake Fyans and Dergholm in the west, large populations of *L. anilitatus* co-occurred with populations of *C. phaeoclavia* and *C. parva*, respectively. In these situations, relatively high proportions of wasps visiting bait flowers were carrying pollinia from the local orchid population, e.g. 30.0, 53.1 and 58.2% at Mullion Creek, Lake Fyans and Dergholm, respectively (Tables 3 and 4). This shows that wasp individuals with previous experience at local flowers were attracted to translocated bait flowers. However, pollination of stigmas was not recorded for pollen-bearing visitors, largely because the behaviour of most wasps was disturbed by lowering an insect net over the flowers in order to capture them before they departed. For this reason, the behavioral data presented here does not necessarily represent the natural situation.

The above results were verified and extended by sequential choice experiments in the field (Table 4). Experiments 1 to 7 compare the responses of *L. anilitatus* to *C. phaeoclavia* and *C. parva* at four sites with high *L. anilitatus* populations, one within the distribution of *C. phaeoclavia* (Mullion Creek, NSW Central Tablelands) and three in the range of *C. parva* (south western Victoria). Translocated *C. phaeoclavia* and *C. parva* attract *L. anilitatus* when exposed sequentially to populations of the wasp in both western Victoria and the NSW Central Tablelands. Some 111 *L. anilitatus* were attracted to *C. parva* and 72 visited *C. phaeoclavia* in the choice tests (Table 4). *L. anilitatus* attempted to mate 14 and 10 times on the labellums of *C. phaeoclavia* and *C. parva* flowers, respectively, and removed pollinia from the anthers of two *C. phaeoclavia* flowers and one *C. parva* flower (Table 4). In addition, nine *L. anilitatus* visited translocated *C. phaeoclavia* flowers from the NSW Central Tablelands carrying pollinia from local *C. parva* plants in western Victoria. Conversely, five *L. anilitatus* carrying pollinia from local *C. phaeoclavia* flowers at Mullion Creek on the NSW Central Tablelands were attracted to translocated *C. parva* flowers from western Victoria (Table 4).

Experiments 8 and 9 (Table 4) focus mainly on *C. villosissima* and *C. parva* in south western Victoria. *C. villosissima* and *C. parva* both attract *L. anilitatus*, with attempted matings on both orchid taxa (Experiment 8, Table 4). *L. anilitatus* from the same wasp population at Dergholm was attracted to 4 samples of *C. parva* from areas close to Dergholm, three samples of *C. parva* from Rosebud, Lake Fyans and the Grampian Ranges, two samples of *C. villosissima* from near Stawell and Langkoop, and a sample of *C. phaeoclavia* from the Central Tablelands of NSW (Experiment 9, Table 4). In addition, all wasp samples contained multiple individuals carrying pollinia from the local population of *C. parva* (Experiment 9, Table 4).

At none of the 42 baiting locations was there a lack of responses by the local population of *L. anilitatus* to any samples of the three *Caladenia* taxa tested at those locations.

Discussion

Caladenia phaeoclavia, C. parva and *C. villosissima* are pollinated by the same common, widespread thynnid wasp species, *Lophocheilus anilitatus*. No evidence was found for cryptic species within *L. anilitatus*. The possession of a shared pollinator by *C. phaeoclavia*, *C. parva* and *C. villosissima* indicates they are likely to have the same allomone mimic of the wasp's sex pheromone (Stökl *et al*. 2005). That the allomone is likely to be identical in all three taxa is supported by the uniform responses of *L. anilitatus* across all wasp populations to all three orchid taxa, including for translocations of up to 750 km.

Strong prezygotic pollinator-mediated reproductive isolation is considered to be characteristic of sexually deceptive orchids (Paulus and Gack 1990, Bower 1996, Xu *et al*. 2011, Whitehead and Peakall 2014). The shared pollinator indicates pollinator-mediated reproductive isolation is absent between the three *Caladenia* species and that they may hybridise where their ranges meet or overlap. Other potential pre-zygotic isolation mechanisms are also absent including differences in pollen position on the vector and flowering times. Hybrids are considered to be relatively common in *Caladenia* (Backhouse and Jeanes 1995) suggesting that postzygotic reproductive barriers to hybridisation in the genus are weak or absent, which is also characteristic of sexual deception in general (Peakall *et al*. 1997, Stökl *et al*. 2008, Vereecken *et al*. 2010, Xu *et al*. 2011, Whitehead and Peakall 2104). The distribution of *C. villosissima* overlaps extensively with that of *C. parva* in western Victoria, and the distributions of *C. phaeoclavia* and *C. parva* meet in southern Victoria and the Grampians, indicating there is a high likelihood of introgression between the three taxa. However, given their close morphological similarity (Backhouse and Jeanes 1995, Jeanes and Backhouse 2006, Fig. 1), hybrids would be very difficult to detect visually in the field.

Although weak postzygotic reproductive isolation seems likely, it cannot be discounted without further study. It is possible, for example, that the three species belong to separate lineages and have converged on the same effective pollinator (Stökl *et al*. 2005). However, the similarity of the three taxa (Fig. 1) suggests they most likely belong to the same lineage, which is reinforced by difficulty in field identification owing to a lack of discrete and definitive morphological differences (Jeanes and Backhouse 2006). A second possibility is that *C. villosissima* may be polyploid, which would create a post-zygotic barrier to hybridization with normal diploid species. *C. villosissima* is a robust species characterised by large leaves, thick stems and a tendency to multiple flowers that is unusual in *Caladenia*. These features suggest polyploid vigour (Comai 2005, Chen 2010) and accordingly *C. villosissima* may be postzygotically isolated. If indeed *C. villosissima* is polyploid, it may co-exist with *C. parva* and *C. phaeoclavia* without hybridizing even though sharing a pollinator with them. The possibility of polyploidy in *C. villosissima* merits study.

If *C. villosissima* is not polyploid, it is considered the available evidence favours the recognition of a single variable pollinator-specific species within the *C. parva* complex. *Caladenia parva* is the name with priority for this complex since it was described first (Carr 1991). *C. parva* in the broad sense would represent a single common, widespread, sexually deceptive orchid species with an abundant, similarly widespread pollinator. This interpretation is consistent with the pollinator data and species definitions that emphasise reproductive isolation and Specific Mate Recognition Systems rather than morphological divergence (Gornall 1997, Coyne and Orr 2004). A similar conclusion was reached for ten pollinator sharing morphospecies in the *Caladenia reticulata* complex (Swartz *et al*. 2014).

The distribution of the *C. parva* complex, encompassing much of the range of the genus *Caladenia* in south-eastern Australia, and its morphological variation, suggest the orchid-thynnid relationship may be of relatively long standing in evolutionary terms. The recognition of three morphospecies within the complex suggests genetic divergence between regions. Accordingly, it would be of interest to determine the genetic variability within the complex and how it compares to other orchid groups, such as the *C. reticulata* complex which comprises many narrow range endemic morphospecies. By contrast many cryptic pollinator-specific sexually deceptive orchids (Bower 1996, 2006; Bower and Brown 1997, Peakall and Whitehead 2013) exhibit few or no morphological differences between sibling species. Such taxa may have diverged relatively recently. Alternatively, they may represent groups with conservative morphology, but high evolutionary lability in allomone composition. In some sexually deceptive orchid lineages, e.g. the *valida* clade in *Chiloglottis* and section *Calonema* of *Caladenia*, there appears to be little selection pressure to change morphology with a change in pollinator, which may reflect the relatively stereotypical mating behaviour of many thynnids (Bower 2006, Bower and Brown 2009).

While it may be argued that the morphological variability in the *C. parva* complex appears unlikely to relate to selection by pollinators, some plant characters may reflect selection by local or regional environmental conditions. If so, local and regional variants may warrant taxonomic recognition, preferably at the subspecies or varietal levels. The *C. parva* complex is highly variable, including within morphospecies, encompassing a wide range of plant heights, flower size, osmophore colouration and tepal positioning. They grow in well-drained shallow stony to skeletal loamy soils and shallow clay loams on the Great Dividing Range in New South Wales, eastern Victoria and the Grampian Ranges, but may grow in stagnant alluvial soils in places like

Lake Fyans, in light brown sandy loams at Dergholm and poorly consolidated sands in southern Victoria. Populations of *C. parva sensu stricto* growing in sand, e.g. Mt. Richmond National Park, have short stems less than 10 cm high and the smallest flowers with deflexed tepals often appressed to the ovary. Although this taxon usually grows in nutrient-deficient soils, the plant form in very sandy habitats may reflect the high nutrient deficiency and low water-holding capacity of this soil type.

While the more extreme variants such as *C. parva* and *C villosissima* may merit continued taxonomic recognition, there are difficulties in maintaining them at any taxonomic level. These include the high probability of hybridisation due to the shared pollinator and the existing identification problems with members of the complex that would continue. Accordingly, it is considered that *C. phaeoclavia* and *C. villosissima* should be synonymized with *C. parva*. Such an approach would recognise the key role that shifts in pollinators play in the speciation of sexually deceptive orchids and the results of recent studies that show species boundaries in cryptic sexually deceptive orchids are defined by allomone chemistry and pollinator specificity (Peakall *et al*. 2010, Whitehead and Peakall 2014). This approach also recognizes the pre-eminence of reproductive isolation in establishing and maintaining species (Coyne and Orr 2004). Reproductive isolation is the underlying rationale of the Biological Species Concept (BSC) (Mayr 1963) and the related Recognition Species Concept (RSC) (Paterson 1995), both of which are highly applicable to sexually deceptive orchids.

Table 1. Pollinator Baiting Locations, Number of *Lophocheilus anilitatus* Wasps Attracted and their Behaviour on Flowers of *C. phaeoclavia, C. parva* and *C. villosissima.* Note: Baiting locations as per Figure 4. Behaviour codes: A = *L. anilitatus* closely approach flowers in flight; C = *L. anilitatus* arrive at bait flowers carrying pollinia from the local population; L = *L. anilitatus* land on bait flowers; M = *L. anilitatus* attempt to mate with bait flowers; P = *L. anilitatus* pollinate bait flowers; R = *L. anilitatus* remove pollinia from bait flowers.

Caladenia species	**Source of orchid sample**			**Lophocheilus anilitatus**		
	Source location name	**Latitude (S)**	**Longitude (E)**	**Baiting locations**	**Number attracted**	**Observed behaviour**
C. phaeoclavia	Albury, NSW	36°03.334'	146°53.546'	B, C2	2	A, L only
	Batlow, NSW	35°31.928'	148°08.235'	MC2	4	A, L only
	Beechworth, Vic	36°19.196'	146°40.159'	B, C2, YM2	22	M, R, C
	Knocker Track, Vic	36°54.909'	147°34.170'	KT	18	M
	Moe, Vic	38°03.002'	146°19.304'	M	10	A, L only
	Mount Jack, Vic	36°29.626'	146°55.561'	B, C2	9	C
	Mullion Creek, NSW (site 1)	33°06.364'	149°04.436'	D, Gr1, Gr2, Gr3, KB, LF, MC1, MK, ML, MR2; YM1,	201	M, R, C, P
	Mullion Creek, NSW (site 2)	33°06.988'	149°10.474'	MC2, PY	6	A, L only
	Nundle, NSW	31°25.746'	151°13.665'	C2, KT, YM2,	27	M, R
	Yetholme, NSW	33°28.920'	149°45.578'	Y1, Y2, YM1, Go	103	M, R
C. parva	Dergholm, Vic (site 1)	37°20.223'	141°14.637'	D, YM2	12	C
	Dergholm, Vic (site 2)	37°19.494'	141°15.526'	D	8	C
	Dergholm, Vic (site 3)	37°17.079'	141°11.168'	D, YM2	23	M, R
	Dergholm, Vic (site 4)	37°16.787'	141°12.988'	D, ML	13	A, L only
	Lake Fyans, Vic	37°08.974'	142°37.057'	CP, D, DGSF, LF, MR1, MR2, Wo, Wi	111	M, R, C
	Mt. Richmond, Vic	38°15.247'	141°25.188'	MC2, MR1, MR2	40	M, R
	Rosebud, Vic	38°22.663'	144°54.311'	D, FI, ML	28	A, L only
	Grampian Ranges, Vic	37°17.121'	142°25.124'	B, D, Gr1, Gr3, LF, MR2, MR2	88	M, C
C. villosissima	Illawarra, Vic (site 1)	37°03.669'	142°41.169'	DGSF	32	M, R
	Illawarra, Vic (site 2)	37°03.943'	142°42.287'	D, YM2	12	A, L only
	Langkoop, Vic	37°07.088'	141°06.039'	D, YM2	13	A, L only
Total					782	

Table 2. Numbers of *Lophocheilus anilitatus* collected at each baiting location.

Baiting Location	Latitude (S)	Longitude (E)	Number of specimens
C. phaeoclavia			
Badja State Forest, NSW	36°02.377′	149°31.585′	17
Beechworth, Vic	36°19.196′	146°40.159′	13
Chiltern, Vic (site 1)	36°15.660′	146°41.953′	4
Chiltern, Vic (site 2)	36°16.511′	146°39.480′	6
Cobobbonee State Forest, Vic	38°12.804′	141°29.094′	12
Dergholm, Vic	37°20.249′	141°14.638′	11
Goulburn, NSW	34°39.929′	149°39.239′	48
Grampian Ranges, Vic (site 1)	37°22.347′	142°30.096′	1
Grampian Ranges, Vic (site 2)	37°16.427′	142°34.839′	10
Grampian Ranges, Vic (site 3)	37°17.121′	142°25.124′	26
Kanangra-Boyd National Park, NSW	33°56.518′	150°03.180′	47
Knocker Track, Vic	36°54.909′	147°34.170′	1
Lake Fyans, Vic	37°08.836′	142°37.012′	13
Millers Landing, Vic	38°55.253′	146°18.160′	10
Moe, Vic	38°03.002′	146°19.304′	24
Mount Kaputar, NSW	30°17.038′	150°09.104′	9
Mullion Creek, NSW (site 1)	33°06.364′	149°04.436′	40
Mullion Creek, NSW (site 2)	33°06.988′	149°10.474′	9
Paling Yards, Orange, NSW	33°21.433′	148°54.712′	1
Yambira Mountain, NSW (site 1)	33°50.342′	148°19.324′	72
Yambira Mountain, NSW (site 2)	33°48.957′	148°20.699′	23
Yetholme, NSW (site 1)	33°24.072′	149°47.728′	4
Yetholme, NSW (site 2)	33°28.920′	149°45.578′	1
C. parva			
Beechworth, Vic	36°19.196′	146°40.159′	6
Cobobbonee State Forest, Vic	38°12.804′	141°29.094′	24
Crib Point, Vic	38°21.681′	145°12.056′	6
Dergholm, Vic	37°20.249′	141°14.638′	77
Devils Garden State Forest, Vic	37°07.822′	142°33.998′	39
French Island, Vic	38°19.848′	145°21.489′	9
Glenelg National Park, Vic	38°09.202′	141°20.802′	1
Grampian Ranges, Vic (site 1)	37°16.427′	142°34.839′	8
Grampian Ranges, Vic (site 3)	37°17.121′	142°25.124′	26
Lake Fyans, Vic	37°08.974′	142°37.057′	51
Millers Landing, Vic	38°55.253′	146°18.160′	13
Mount Richmond National Park, Vic	38°15.550′	141°25.9683′	15
Mullion Creek, NSW	33°06.364′	149°04.436′	27
Wilkin Flora and Fauna Reserve, Vic	37°40.335′	141°13.878′	6
Woohlpooer, Vic	37°17.208′	142°07.308′	10
Yambira Mountain, NSW (site 1)	33°49.230′	148°20.513′	5
C. villosissima			
Dergholm, Vic	37°20.249′	141°14.638′	20
Devils Garden State Forest, Vic	37°07.710′	142°34.142′	32
Yambira Mountain, NSW (site 1)	33°49.230′	148°20.513′	5
Total			**782**

Table 3. Behavioural interactions of *Lophocheilus anilitatus* on flowers of three species of Greencomb Spider Orchids (number).

	Eastern NSW and Victoria				South Western Victoria			
***Caladenia* species**	**Attempted matings**	**Pollinia removal**	**Pollination of stigma**	**Wasps arriving with pollinia**	**Attempted matings**	**Pollinia removal**	**Pollination of stigma**	**Wasps arriving with pollinia**
C. phaeoclavia	54	13	2	21	43	2	0	9
C. parva	1	1	0	6	24	4	0	80
C. villosissima	0	0	0	0	3	1	0	10

Table 4. Attraction of *Lophocheilus anilitatus* by *Caladenia phaeoclavia, C. parva* and *C. villosissima* in field choice tests. Refer 'Methods' for explanation of test procedures. Flowers labelled 'A' were exposed to pollinators in the first three minutes; flowers labelled 'B' and subsequent letters were exposed for subsequent three minute intervals at the same sites, either with sample A (Experiments 1, 3 and 8) or without previous samples (Experiments 2, 4, 6, 7 and 9). Experiment 9 was conducted with the assistance of Australasian Native Orchid Society members and not all behavioural data was recorded. NR = not recorded.

***Caladenia* species**	**Source of orchid**	**No. 3 min. exposures**	**No. wasps caught**	**No. attempted matings**	**No. pollinaria removals**	**No. visitors with pollinaria**
Experiment 1. Mullion Creek (site 1), NSW, 7 October 1995, Sequential test.						
A. *C. parva*	Lake Fyans, Vic.	30	7	0	0	0
B. *C. phaeoclavia*	Mullion Creek (site 1), NSW	30	3	0	0	0
Experiment 2. Mullion Creek (site 1), NSW, 14 October 1995, Sequential single species test.						
A. *C. parva*	Mt. Richmond National Park, Vic	10	12	0	0	5
B. *C. phaeoclavia*	Mullion Creek (site 1), NSW	14	8	0	0	1
Experiment 3. Grampian Ranges (site 1), Vic, 22 Oct. 1996, Sequential test.						
A. *C. phaeoclavia*	Mullion Creek (site 1), NSW	9	26	7	1	1
B. *C. parva*	Grampian Ranges (site 2), Vic	9	11	1	0	0
Experiment 4. Grampian Ranges (site 2), Vic, 22 Oct. 1996, Sequential single species test.						
A. *C. phaeoclavia*	Mullion Creek (site 1), NSW	16	10	4	1	0
B. *C. parva*	Grampian Ranges (site 1), Vic	13	8	1	0	0
Experiment 5. Lake Fyans, Vic, 23 Oct. 1996, Sequential single species test.						
A. *C. parva*	Lake Fyans, Vic.	12	19	1	0	12
B. *C. phaeoclavia*	Mullion Creek (site 1), NSW	11	13	2	0	8
C. *C. parva*	Grampian Ranges (site 1), Vic	4	17	0	0	6
Experiment 6. Cobobbonee State Forest, Vic, 24 Oct. 1996, Sequential single species test.						
A. *C. parva*	Grampian Ranges (site 1), Vic	1	11	4	0	0
B. *C. phaeoclavia*	Mullion Creek (site 1), NSW	1	12	1	0	0
C. *C. parva*	Mt. Richmond National Park, Vic	5	13	3	1	0
Experiment 7. Mullion Creek (site 1), NSW, 26 Oct. 1996, Sequential single species test.						
A. *C. parva*	Grampian Ranges (site 1), Vic	7	10	0	0	0
B. *C. parva*	Mt. Richmond National Park, Vic	12	3	0	0	0
Experiment 8. Devils Garden State Forest, Vic, 11 Oct. 2006, Sequential test.						
A. *C. villosissima*	Illawarra (site 1), Vic	7	27	3	1	0
B. *C. parva*	Lake Fyans, Vic	7	20	2	0	0
Experiment 9. Dergholm (site 2), Vic, 16 Oct. 2008, Sequential single species test.						

A. *C. parva*	Dergholm (site 1), Vic	11	10	0	0	4
B. *C. parva*	Dergholm (site 2), Vic	14	8	0	0	6
C. *C. parva*	Dergholm (site 3), Vic	NR	20	NR	NR	16
D. *C. parva*	Dergholm (site 4), Vic	NR	10	NR	NR	4
E. *C. parva*	Grampian Ranges (site 1)	NR	10	NR	NR	3
F. *C. parva*	Rosebud, Vic	NR	9	NR	NR	7
G. *C. parva*	Lake Fyans, Vic	NR	11	NR	NR	7
H. *C. villosissima*	Langkoop, Vic	NR	10	NR	NR	7
I. *C. villosissima*	Illawarra (site 2), Vic	NR	10	NR	NR	3
J. *C. phaeoclavia*	Mullion Creek (site 1), NSW	NR	11	NR	NR	NR

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