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Steve John Wylie * , Hua Li , Shu Hui Koh

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A Survey of Wild Indigenous *Cryptostylis ovata* Orchid Populations in Western Australia Reveals Spillover of Exotic Viruses

Steve Wylie [1,*], Hua Li [1] and Shu Hui Koh [2]

[1] Food Futures Institute, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia
[2] School of Medical, Molecular and Forensic Sciences, College of Environmental and Life Sciences, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia
* Correspondence: s.wylie@murdoch.edu.au

Abstract: *Cryptostylis ovata* is a terrestrial orchid endemic to southwestern Australia. The virus status of *C. ovata* has not been studied. Eighty-three *C. ovata* samples from 16 populations were collected, and sequencing was used to identify RNA viruses in them. In one population, all tested plants were co-infected with isolates of the exotic-to-Australia viruses Ornithogalum mosaic virus (OrMV) and bean yellow mosaic virus (BYMV). In another population, one plant was infected with BYMV. No viruses were detected in the remaining populations. The OrMV isolate shared 98–99% nucleotide identities with isolates identified from wild indigenous *Lachenalia* (Iridaceae) plants in South Africa. This suggests that the source of OrMV in *C. ovata* may be one or more of bulbous iridaceous flowering plants of southern African origin that were introduced to Western Australia as ornamentals and that have since become invasive weeds. One BYMV isolate from *C. ovata* also exhibited 99% nucleotide identity with strains isolated from exotic leguminous crops such as *Lupinus angustifolius* in Western Australia, suggesting spillover to indigenous species from these sources. This study with *C. ovata* highlights the probable role of invasive weeds and exotic crops as sources of exotic virus spillovers to indigenous plants.

Keywords: virus emergence; wild-plant virology; virus transmission; potyvirus

1. Introduction

Australia is a global center of orchid biodiversity. Of the 29,524 accepted orchid taxa [1], approximately 1,700 are indigenous to Australia [2]. Wild orchids face numerous threats, primarily habitat loss due to human activities and climate change. Additional threats include plant removal for collections, the decline of symbiotic pollinator species and mycorrhizal fungi, destruction by animals, competition from invasive weeds, and damage caused by pests and diseases [3–5].

The genus *Cryptostylis* comprises 23 species of orchids with a predominantly Southern Hemisphere distribution. The greatest number of species occurs in the Philippines, Indonesia and Papua New Guinea (World Checklist of Vascular Plants, Royal Botanic Gardens, Kew, 2024). Genetic analysis of *Cryptostylis* species from Australia and Asia supports an Australian origin for the genus, followed by a single dispersal event to Asia and subsequent speciation [6]. Of the five *Cryptostylis* species indigenous to Australasia, four are found in eastern Australia and New Zealand, while one, *Cryptostylis ovata*, is confined to southern Western Australia [7]. Notably, *C. ovata* is the only evergreen orchid among the approximately 400 terrestrial orchid taxa indigenous to Western Australia; all others are deciduous, surviving much of the year as underground bulbs or tubers.

Viruses infecting members of the Orchidaceae in Australia, as with the flora generally, are poorly studied. While many virus species identified from Australian orchids appear to have evolved on the continent, only *Divavirus* and *Platypuvirus* represent genera that may be endemic to Australia. Most other apparently indigenous viruses identified from Australian orchids belong to internationally distributed

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genera, such as *Potyvirus*. Examples include *Diuris virus Y* [8] (*Potyvirus*), *Donkey orchid virus A* [9] (*Potyvirus*), *Caladenia virus A* [9] (*Poacevirus*), and *Pterostylis blotch virus* [10] (*Orthotospovirus*).

In addition to indigenous viruses, several Australian orchids are infected with exotic viruses. These viruses, which originate outside Australia, were likely introduced over the past two centuries through plants imported for agriculture, horticulture, and landscaping, and from weeds. Exotic viruses have since spilled over into native orchids. For example, isolates of bean yellow mosaic virus (BYMV, *Potyvirus*) and Ornithogalum mosaic virus (OrMV, *Potyvirus*) were identified in potted *Diuris magnifica* orchids in Perth, W.A., and in wild *D. corymbosa* orchids in remnant bushland near Brookton, Western Australia [11]. In eastern Australia, BYMV has been detected in *Pterostylis curta* and *Diuris* species orchids, while OrMV, (referred to as *Pterostylis virus Y* but later confirmed to be OrMV) was identified from species of *Pterostylis*, *Chiloglottis*, *Diuris*, *Eriochilus*, and *Corybas* orchids [8].

To date, no *Cryptostylis* species has been investigated for the presence of viruses. In this study, we tested leaf samples from wild populations of *C. ovata* for RNA viruses using a high-throughput sequencing approach. This method is advantageous over other virus detection techniques such as PCR- and antibody-based approaches as it does not require prior knowledge of the viruses being investigated.

2. Materials and Methods

Leaf samples were collected from 83 individual *C. ovata* plants from 16 populations in southern Western Australia (Table 1). Due to the lateral spread of *C. ovata* plants through rhizomes, it was challenging to delineate individual plants. To address this, leaves spaced at least 1 m apart were considered to belong to distinct individual plants. Sampling sites included a variety of habitats, ranging from remnant roadside bushland to indigenous forests and exotic *Pinus radiata* plantations. The aim was to collect samples from 10 individuals per population; however, in populations with fewer than 10 plants, one sample was collected from every available plant. Samples were taken regardless of visible symptoms of virus infection, such as chlorosis on young leaves, mosaic patterns, necrosis, or stunting.

Table 1. *Cryptostylis ovata* populations tested.

Population	Samples	Latitude	Longitude	Site type
Abba Rd, Yoganup	5	-33.774722	115.572056	Eucalyptus mixed woodland
Green Hill Rd, Augusta	3	-34.29466	115.13329	Eucalyptus mixed woodland
Bowelling-Duranillin Rd, Wunnerberg	8	-33.43865	116.51011	Road verge remnant mixed woodland
Johnson Rd, Bertram	6	-32.240827	115.848103	Eucalyptus mixed woodland
Brockman Hwy, Darradup	3	-34.151395	115.504124	Pine plantation
Inlet Dr, Denmark	1	-34.990129	117.355263	Eucalyptus mixed woodland
Devlin Rd, Wellesley	6	-33.223689	115.771459	Road verge, remnant mixed woodland
Forestry Rd, Uduc	1	-33.064971	115.789744	Road verge remnant mixed woodland
Gray Rd, Boyanup	7	-33.475876	115.757871	Road verge remnant mixed woodland
Nettleton Rd, Jarrahdale	7	-32.323770	116.070251	Eucalyptus mixed woodland
Lakes Rd, Dalyellup	10	-33.439839	115.608363	Eucalyptus mixed woodland
Coolilup, Ludlow	5	-33.608771	115.499426	Pine plantation
Windy Harbour Rd, Mt Chudalup	3	-34.765220	116.084133	Eucalyptus mixed woodland
Crockerup Rd, Mt Barker	1	-34.572150	117.672821	Road verge remnant mixed woodland
Sabina Rd, Yoganup	7	-33.739220	115.499528	Pine plantation
Woods Rd, Gelorup	10	-33.430819	115.623445	Road verge mixed woodland

Leaf samples were collected from 83 individual *C. ovata* plants from 16 populations in southern Western Australia (Table 1). Due to the lateral spread of *C. ovata* plants through rhizomes, it was challenging to delineate individual plants. To address this, leaves spaced at least 1 m apart were considered to belong to distinct individual plants. Sampling sites included a variety of habitats, ranging from remnant roadside bushland to indigenous forests and exotic *Pinus radiata* plantations. The aim was to collect samples from 10 individuals per population; however, in populations with fewer than 10 plants, one sample was collected from every available plant. Samples were taken regardless of visible symptoms of virus infection, such as chlorosis on young leaves, mosaic patterns, necrosis, or stunting.

Total RNA was extracted from 100 mg of leaf tissue using the RNeasy Plant Mini Kit (Qiagen) after grinding the samples in liquid nitrogen. To prepare for cDNA library construction, ribosomal

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RNA was depleted using the Ribo-Zero Plant kit, and libraries were generated with the TruSeq Stranded Total RNA Plant Library preparation kit (Illumina). Paired-end high-throughput sequencing (HTS) was performed on an Illumina NovaSeq 6000 S4 platform with 150 cycles.

Post-sequencing, TruSeq primer-adaptors were removed, and quality trimming was performed using default parameters in CLC Genomics Workbench (Qiagen). Reads shorter than 100 nucleotides were discarded. *De novo* assembly was conducted in both CLC Genomics Workbench and Geneious Prime (Biomatters). Contigs longer than 500 nucleotides were analysed using Blastn. Contigs matching viral sequences and those with no matches (referred to as orphans) were subjected to further analysis.

Orphan contigs were translated in six reading frames (three forward and three reverse). Contigs without open reading frames (ORFs) of at least 100 amino acid residues were discarded. For those with ORFs, nucleotide and amino acid sequences were analysed in Blastn or Blastp, respectively, for similarities to known sequences.

Based on HTS results, five sets of species-specific primers were synthesised for each identified virus (Table S1). Primers were designed in Primer 3, each with a melting temperature of 60°C.

All 83 RNA samples were then tested using these primers via RT-PCR. Amplified bands were sequenced using the Sanger method. Primer sequences were trimmed from the resulting sequences, which were then aligned against HTS-derived sequences and publicly-available sequences from GenBank using Blastn. Gaps between amplicons were filled by combining appropriate primers and performing additional Sanger sequencing. This process enabled the identification of virus-derived sequences and facilitated the assembly of complete or partial genome sequences for each virus present in infected plants.

3. Results

3.1. Plant Samples

In each population except the Bowelling-Duranillin Road population, plants appeared to be healthy, lacking symptoms typical of virus infection. At the Bowelling-Duranillin Road population, all eight plants displayed mosaic patterns and chlorotic streaking symptoms reminiscent of virus infection (Figure 1).

Figure 1. *Cryptostylis ovata* plants of the Bowelling-Duranillin Road, Wunnerberg population where virus-like symptoms of yellow streaks and mosaic patterns occurred on leaves (left). Symptomless *C. ovata* leaves of the Coolilup population (right).

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3.2. Viruses

Following RNA sequencing, viruses were identified from two *C. ovata* populations. The eight samples tested from the Bowelling-Duranillin Road population were doubly-infected with bean yellow mosaic virus (BYMV) and Ornithogalum mosaic virus (OrMV). One of the six plants from the Devlin Rd population was infected with an isolate of BYMV. No viruses were identified from the other *C. ovata* plants tested.

3.3. Sequence Analysis

Alignment of the eight BYMV sequences from the Bowelling-Duranillin Road population revealed that they were identical. This isolate was named BYMV-BDW. One of the samples collected from the Devlin Rd population was infected with a distinct isolate of BYMV. (Figure 2).

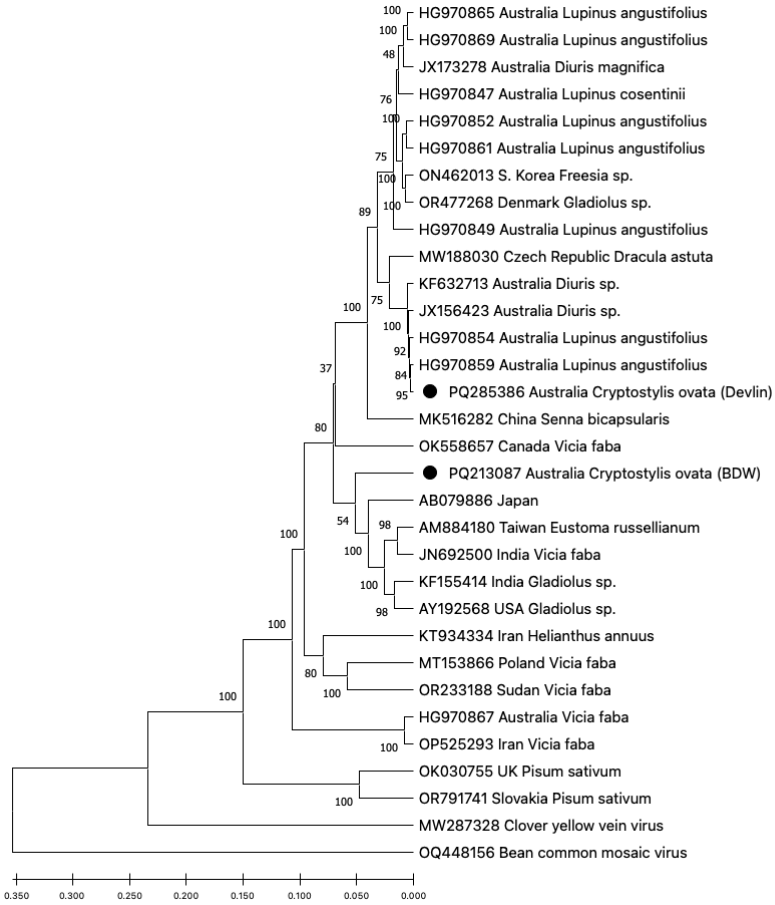

Figure 2. Phylogenetic tree showing genetic relationships of the large coding region (nucleotides) of bean yellow mosaic virus (BYMV) isolates Devlin and BDW from *Cryptostylis ovata* plants (indicated by black dots) compared with those of other BYMV isolates. Maximum Likelihood was used to infer evolutionary relationships. GenBank accessions are shown, followed by the country of isolation and host species for most isolates, the two exceptions being isolates of bean common mosaic virus and clover yellow vein virus, used as outgroups, where country of isolation and host species are not provided. Genetic distances were calculated using Maximum Likelihood and units are base substitutions per site. One thousand replications were done for each analysis and percentage support is shown at nodes.

The eight OrMV sequences from the the Bowelling-Duranillin Road population were identical, and this isolate was named OrMV-BDW (Figure 3).

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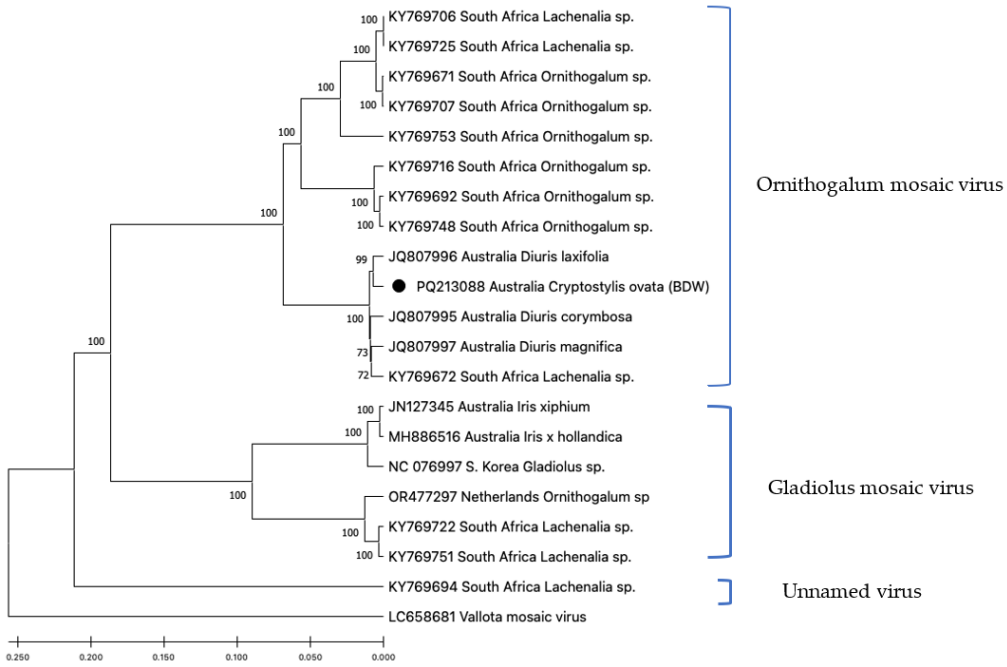

Figure 3. Phylogenetic tree showing genetic relationships of the genome (nucleotides) of Ornithogalum mosaic virus (OrMV) isolate BDW from *Cryptostylis ovata* plants (indicated by black dot) compared with those of other OrMV isolates. Maximum Likelihood was used to infer evolutionary relationships. GenBank accessions are shown, followed by the country of isolation and host species for most isolates. Isolates of Gladiolus mosaic virus (some previously named Ornithogalum mosaic virus), an unnamed virus and vallota mosaic virus, are provided as outgroups. Genetic distances were calculated using Maximum Likelihood and units are base substitutions per site. One thousand replications were done for each analysis and percentage support is shown at nodes.

The complete genome sequence of BYMV-BDW was 9481 nt, encoding 3056 aa, while isolate BYMV-Devlin was incomplete at 8387 nt at the 5′ end, encoding 2749 aa and lacking the 5′ untranslated region and the terminal region of the P1 gene. Isolates BYMV-BDW and BYMV-Devlin were not identical, sharing 91% nt and 96% aa identities over the common regions. Phylogenetic analysis of the nucleotide sequences of these two isolates with other BYMV genome sequences available from GenBank revealed that BYMV-Devlin shared 99% identities with four other BYMV isolates from W.A., two from wild symptomatic *Diuris* orchids and two from *Lupinis angustifolius* (narrow-leafed lupin) plants collected from crops. In contrast, BYMV-BDW was not closely aligned with other BYMV isolates from Australia, instead sharing up to 92% identity with BYMV isolates from India, Taiwan, Japan and the USA collected from diverse hosts (Figure 2).

The complete genome sequence of isolate OrMV-BDW was 9045 nt. OrMV-BDW shared greatest identities (98-99%) with OrMV isolates identified from indigenous *Diuris* orchids from Western Australia, and also with an isolate from a wild *Lachenalia* sp. plant collected in South Africa, where it is an indigenous species (Figure 3).This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

4. Discussion

This paper presents the first report of viruses infecting plants of the orchid *C ovata.* Of the sixteen *Cryptostylis ovata* populations tested, only two populations had plants infected with one or two viruses. The two viruses, BYMV and OrMV, have origins outside Australia. No indigenous viruses were identified.

Interestingly, the eight plants collected from the Bowelling-Duranillin Road population were all infected with identical isolates of BYMV and OrMV. This suggests that this population may either

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comprise a single large plant that has spread several metres in diameter, or it may comprise individuals infected with the same virus isolates through aphid vectors or through root or leaf contact.

BYMV has a broad international distribution and host range, infecting both monocotyledonous and dicotyledonous plants over all temperate cropping regions. It poses a serious threat to leguminous crops, such as narrow-leafed lupins and clover/medic pastures in Western Australia [12,13] and elsewhere, as well as to floriculture crops such as *Gladiolus* sp. in several countries [14,15]. BYMV has been reported from a range of wild and cultivated orchids, including *Vanilla* and other orchids in French Polynesia [16], *Masdevallia* orchids from the USA, and *Calanthe* orchids from Japan [17]. Like other potyviruses, BYMV is transmitted horizontally by aphids, mechanically by physical contact, and via pollen, and vertically through seed in several plant species [18,19], although seed transmission has not been recorded in orchids.

No aphids are reported infesting the leaves of *C. ovata*, and, indeed, these authors have not observed this in decades of observation. However, the authors have noted aphids colonizing flowers, suggesting this may be the route through which aphids transmit viruses from source plants to *C. ovata*. Notably, *C. ovata* produces flowers in mid-summer, after some major BYMV sources, such as narrow-leafed lupin crops, have already been harvested in Western Australia.

BYMV is a genetically diverse virus, with several groupings proposed based on nucleotide sequence phylogeny and host preferences [20–22]. BYMV-Devlin shares greatest genetic identity with isolate BYMV-SW3.2 already described from Western Australia in wild orchids (JX156423) (Figure 2), suggesting this strain is widespread among different hosts in the region as these two host orchids were located at least approximately 140 km apart. In the *Diuris corymbosa* orchid source, BYMV-SW3.2 infection caused chlorotic leaf mottle [11], while the *C. ovata* plant infected with the near-identical isolate BYMV-Devlin appeared asymptomatic. In contrast, plants infected with BYMV-BDW exhibited strong symptoms of infection. Since Koch's postulates were not applied, it remains unclear whether the pronounced symptoms in the Bowelling-Duranillin Road population were caused by BYMV-BDW, OrMV-BDW, or both. The BYMV-BWD sequence closely resembled the *Gladiolus hybrida* isolate BYMV-M11 (AB079886) from Japan, which induced mild symptoms or no symptoms in *Nicotiana benthamiana* and *Vicia faba* plants [23]. It is also possible that there are non-viral origins for the symptoms observed in the Bowelling-Duranillin Road population.

Like BYMV, OrMV is an aphid-transmitted potyvirus with a broad geographical and host range, but it appears to be restricted to monocotyledonous plants. It is a significant pathogen of floricultural crops, especially ornamental bulbous plants originating from Africa, such as *Iris*, *Ornithogalum*, *Lachenalia*, *Sparaxis*, *Gladiolus*, *Tritonia*, and others [24]. OrMV was first identified in Western Australia from ornamental *Iris* plants in home gardens [9]. Species of several iridaceous genera, such as *Gladiolus*, *Homeria*, *Ixia*, *Lachenalia*, and *Freesia* of southern African origin have become naturalised weeds in Western Australian bushlands [25]. Their role as hosts for OrMV and BYMV remains untested.

The presence of close-to-identical (99%) OrMV sequences in indigenous *Diuris* orchid populations in Western Australia located approximately 125 km from the BDW population, and an *Iris* plant from a domestic garden in Western Australia, located approximately 230 km from the BDW population, suggest there has been a single introduction of OrMV into Western Australia, but more extensive sampling is required to confirm this. The high genetic identity (98%) between the Western Australia OrMV isolates and an isolate from a *Lachenalia* plant in South Africa indicates that bulbous ornamental plants imported into Western Australia as garden plants, including several *Lachenalia species* [26], are sources of OrMV to the region. More study is required to determine if such weeds continue to host OrMV and the means by which this virus is transmitted between them, whether by feed aphid vectors or pollen carried by bees or the wind.

This study demonstrates that spillover of two exotic potyviruses to the indigenous *Cryptostylis ovata* orchid has occurred in Western Australia. The probable origin of OrMV lies in invasive weeds introduced into Australia as bulbous ornamental garden plants from Africa, and which are now rapidly invading bushlands. The origin of BYMV could be in seeds of leguminous crop and pasture species, as well as in invasive garden plants. Invasive weeds not only compete with indigenous species for resources such as space, water and nutrients, but also likely serve as reservoirs for

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damaging viruses. Further research is needed to understand the roles of such weeds in viral spillovers to indigenous species, including orchids, in Australian bushlands.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Primer pairs used to confirm presence of bean yellow mosaic virus (primer names beginning BYMV) and Ornithogalum mosaic virus (primer names beginning OrMV) by PCR. The primer names also contain information on the name of the genes to which they bind and whether they are forward (F) or reverse (R) primers.

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References

1. Pérez-Escobar, O.A.; Bogarín, D.; Przelomska, N.A.; Ackerman, J.D.; Balbuena, J.A.; Bellot, S.; Bühlmann, R.P.; Cabrera, B.; Cano, J.A.; Charitonidou, M. and Chomicki, G. The origin and speciation of orchids. *New Phytologist* **2024**, *242*, 700-716.
2. Jones DL (2024) A complete guide to native orchids of Australia. Revised 3rd edn. New Holland Publishers, Sydney, Australia, 2024; pp. 12-54.
3. Swarts, N.D.; Dixon, K.W. Terrestrial orchid conservation in the age of extinction. *Ann bot* **2009**, *104*, 543-556.
4. Brundrett, M.C. Using vital statistics and core-habitat maps to manage critically endangered orchids in the Western Australian wheatbelt. *Aust J Bot* **2016**, *64*, 51-64.
5. Kolanowska, M.; Michalska, E. The effect of global warming on the Australian endemic orchid *Cryptostylis leptochila* and its pollinator. *Plos One* **2023**, *18*(1), p.e0280922.
6. Weinstein, A.M. Pollination ecology of Australian sexually deceptive orchids with contrasting patterns of pollinator exploitation. PhD, The National University of Australia, Canberra, 2021.
7. Arifin, A.R.; Phillips, R.D.; Weinstein, A.M.; Linde, C.C. *Cryptostylis* species (Orchidaceae) from a broad geographic and habitat range associate with a phylogenetically narrow lineage of Tulasnellaceae fungi. *Fung Biol* **2022**, *126*, 534-546.
8. Gibbs, A.; Mackenzie, A.; Blanchfield, A.; Cross, P.; Wilson, C.; Elliot, K.; Nightingale, M.; Clements, M. Viruses of orchids in Australia: Their identification, biology and control. *Aust Orchid Rev* **2000**, 65, 10–21
9. Wylie, S.J.; Tan, A.J.Y.; Li, H.; Dixon, K.W.; Jones, M.G.K. Caladenia virus A, an unusual new member of the family *Potyviridae* from terrestrial orchids in Western Australia. *Arch Virol* **2012**, *157*, 2447–2452.
10. Chao, H.Y.; Clements, M.A.; Mackenzie, A.M.; Dietzgen, R.G.; Thomas, J.E.; Geering, A.D. Viruses infecting greenhood orchids (Pterostylidinae) in Eastern Australia. *Viruses* **2022**, *14*, 365.
11. Wylie, S.J.; Li, H.; Dixon, K.W.; Richards, H.; Jones, M.G.K. Exotic and indigenous viruses infect wild populations and captive collections of temperate terrestrial orchids (*Diuris* Species) in Australia. *Virus Res* **2013**, 171, 22–32.
12. Cheng, Y.; Jones, R.A.C. Distribution and incidence of necrotic and non-necrotic strains of bean yellow mosaic virus in wild and crop lupins. *Aust J Agric Res* **1999**, *50*, 589-600.
13. Jones, R.A. Virus diseases of perennial pasture legumes in Australia: incidences, losses, epidemiology, and management. *Crop Past Sci* **2013**, *64*, 199-215.

 doi:10.20944/preprints202412.0963.v1

14. Katoch, M.; Ram, R.; Zaidi, A.A.; Garg, I.D. Status of bean yellow mosaic virus on *Gladiolus*. *Crop Prot* **2002**, *21*, 861-865.
15. Kaur, C.; Raj, R.; Srivastava, A.; Kumar, S.; Raj, S.H.K. Sequence analysis of six full-length bean yellow mosaic virus genomes reveals phylogenetic diversity in India strains, suggesting subdivision of phylogenetic group-IV. *Arch Virol* **2018**, *163*, 235-242.
16. Agronomique, P.; Tahiti, F.P. Virus infections of *Vanilla* and other orchids in French Polynesia. *Plant Dis* **1987**, *71*, 1125.
17. Hammond, J.; Lawson, R.H. A strain of bean yellow mosaic virus is aphid-transmitted from orchid. In Proceedings of the VII International Symposium on Virus Diseases of Ornamental Plants, Sanremo, Ital, (29 May 1988).
18. Pathipanowat, W.; Jones, R.A.C.; Sivasithamparam, K. Studies on seed and pollen transmission of alfalfa mosaic, cucumber mosaic and bean yellow mosaic viruses in cultivars and accessions of annual *Medicago* species. *Aust J Agric Res* **1995**, *46*, 153-165.
19. Sasaya, T.; Iwasaki, M.; Yamamoto, T. Seed transmission of bean yellow mosaic virus in broad bean (*Vicia faba*). *Japan J Phytopathol* **1993**, *59*, 559-562.
20. Wylie, S.J.; Coutts, B.A.; Jones, M.G.K.; Jones, R.A.C. Phylogenetic analysis of bean yellow mosaic virus isolates from four continents: relationship between the seven groups found and their hosts and origins. *Plant Dis* **2008**, *92*, 1596-1603.
21. Kehoe, M.A.; Coutts, B.A.; Buirchell, B.J.; Jones, R.A. Split personality of a potyvirus: to specialize or not to specialize?. *PLoS One* **2014**, *9*(8), p.e105770.
22. Kaur, C.; Raj, R.; Kumar, S.; Purshottam, D.K.; Agrawal, L.; Chauhan, P.S. and Raj, S.H.K. Elimination of bean yellow mosaic virus from infected cormels of three cultivars of *Gladiolus* using thermo-, electro-and chemotherapy. *3 Biotech* **2019**, *9*, 1-10.
23. Nakazono-Nagaoka, E.; Sato, C.; Kosaka, Y. Evaluation of cross-protection with an attenuated isolate of bean yellow mosaic virus by differential detection of virus isolates using RT-PCR. *J Gen Plant Pathol* **2004**, *70*, 359–362.
24. Gao, F.; Du, Z.; Shen, J.; Yang, H.; Liao, F. Genetic diversity and molecular evolution of Ornithogalum mosaic virus based on the coat protein gene sequence. *Peer J* **2018**, *6*, p.e4550.
25. Stenhouse, R.N. Fragmentation and internal disturbance of native vegetation reserves in the Perth metropolitan area, Western Australia. *Landsc Urb Plan* **2004**, *68*, 389-401.
26. Brown, K.; Brooks, K.; Madden, S.; Marshall, J. Control of the exotic bulb, Yellow Soldier (*Lachenalia reflexa*) invading a *Banksia* woodland, Perth, Western Australia. *Ecol Manag Restor* **2002**, *3*, 28-36.