Bringing Australia's vulnerable *Wittsteinia vacciniacea* F.Muell. (Baw-Baw berry) into cultivation

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Abstract

Wittsteinia vacciniacea F.Muell. (Baw-Baw berry) is a vulnerable evergreen trailing shrub dating back over 70 million years, when Australia separated from Gondwanaland during the late Cretaceous period. It is the only species of *Wittsteinia* to occur in Australia, and one of four genera in Alseuosmiaceae. The Baw-Baw berry is restricted to a few mountainous populations of varying size and occupancy within Victoria. The fragrant flowers are attractive pendent yellow-red bells, and the globose fruit a greenish-red berry with persistent attractive calyx lobes. It is the combination of a trailing habit and floral features that make this species an ideal candidate for amenity horticulture. To test this, we collected plant material from five of the six known localities in Victoria and, using stem/cutting material with +/- Clonex® gel and either Jiffy® Plugs or a perlite/vermiculite mix +/- Clonex®, achieved 60.8 per cent root initiation overall. Greater root development was achieved using the plugs, and there were differences between root initiation and species locality. The hormone treatment proved unnecessary to ensure root initiation. To test germination response, seed was collected from Mt Baw Baw, which supports the largest population. Preliminary work on fresh seed indicated a high fill rate, determined by x-ray imagery, and a 90 per cent germination response at 20/15 °C with gibberellic acid (GA_3). We explored the germination niche using a thermogradient plate and determined an optimal temperature of ~17 °C. Resulting vegetative and seed-based propagules have been incorporated into the Royal Botanic Gardens Victoria living collection, as part of a broader *ex situ* conservation strategy. Further to this, using predictive modelling, we found areas outside this species' current distribution that may be suitable for future plantings, ensuring the Baw-Baw berry's survival beyond its current refugia.

Introduction

Plant and fungal biodiversity support all life on Earth yet, alarmingly, 39 per cent of all vascular plant species are threatened with extinction (Lughadha *et al.*, 2020). Conservation horticulture is an emerging discipline driven by botanic gardens working towards global strategies to address plant

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conservation, often with an emphasis on *ex situ* living plant collections of high preservation value. Considered a conduit position, the role brings together the ecology of a threatened species with the horticultural techniques to successfully propagate and provide subsequent plant material for *ex situ* living collections, reintroduction or, more broadly, cultivation. *Wittsteinia vacciniacea* F.Muell. (Baw-Baw berry) is a strong candidate to test conservation horticultural methods as it is a vulnerable subalpine species (DELWP, 2023), with a distribution limited to a few mountainous areas of Victoria, Australia. It is one of the three species of *Wittsteinia*, the others being *W. balansae* (Baill.) Steen. in New Caledonia and *W. papuana* (Steen.) Steen. in Papua New Guinea. They are members of the very small family Alseuosmiaceae, which only occurs in Australia, New Zealand, New Caledonia and Papua New Guinea. The family also includes the genera *Crispiloba* (a monotypic genus from Queensland, Australia), *Alseuosmia* (containing five species, all from New Zealand's North Island) and *Platyspermation*, a monotypic genus from New Caledonia. Alseuosmiaceae is known for its ancient Gondwana flora, thought to be in existence for the last 70 million years (Wikström *et al*., 2001). It is therefore fundamental to safeguard against any potential evolutionary loss regarding such a unique relict plant.

In May 2021, *Wittsteinia vacciniacea* was listed as Vulnerable under the Victorian State Flora and Fauna Guarantee Amendment Act, 2019. Fire frequency and intensity were both deemed high risks for the species given its lack of effective post-fire response strategies (DELWP, 2022). This risk is compounded by the added likelihood of the current sub-alpine habitat drying out due to climate change, increased soil erosion, competition from invasive weeds and the risk of predation from feral deer (Quin, 2021; DELWP, 2022). The combined impacts of these threatening processes highlight the potential value that conservation horticulture could have for safeguarding the species.

The growth form of *Wittsteinia vacciniacea* lends itself to a horticultural application as a groundcover plant – it is strikingly similar to *Pachysandra terminalis* Siebold & Zucc., an established horticultural plant used as groundcover, but without the fungal blight problems that beset the latter. *W. vacciniacea* is a creeping subshrub with abundant adventitious roots (Dickison, 1986), rooting readily at nodes and growing to approximately 40 cm high, with a reasonably restricted spread. The flowers, which are lightly scented, bell-shaped and flare at the tips, appear in late spring to early summer. The fruit is globose in shape with attractive and persistent calyces (Fig. 1).

The structural protection that *Wittsteinia vacciniacea* provides via its partly recumbent growth habit (Fig. 2) allows for the movement, calling sites and breeding micro-habitat of the Critically Endangered Baw Baw frog (*Philoria frosti*) (IUCN, 2023; Hollis, 2004). Baw Baw frogs are endemic to the Baw Baw Plateau and are Victoria's only endemic amphibian. It is estimated that < 1,000 frogs remain in the wild (Marshall, 2022). Given the known associations between this frog species and *W. vacciniacea*, conservation strategies will likely have far-reaching benefits beyond the conservation of the species itself.

To our knowledge, there is no published work on the propagation, seed viability and germination of *Wittsteinia vacciniacea*, or on the other two species within the genus. Due to the vulnerability of *W. vacciniacea* and its relict place in Australia's Gondwana flora, it

Fig. 1 *Wittsteinia vacciniacea* (Baw-Baw berry) flowers (left) and fruit (right). Note the calyces which persist from flower to fruit. Photos: V. Williamson.

Fig. 2 Growth habit of *Wittsteinia vacciniacea* (right-hand side of rock) under snow gums (*Eucalyptus pauciflora* subsp. *acerina*) at Mt Baw Baw, Victoria, Australia. Photo: V. Williamson.

should be propagated to ensure its survival and evaluated for its horticultural potential. Assessing the viability and germination of *W. vacciniacea* for future seed reserves in *ex situ* conservation seed banks is also an important consideration. Successful *ex situ* conservation will also open opportunities for enhanced *in situ* conservation measures and, through environmental niche modelling, will potentially identify suitable translocation sites.

Materials and methods

Vegetative plant material of *Wittsteinia vacciniacea* was collected from the five known accessible sites in November (spring) 2022 (Table 1; a map of current locations is shown in Fig. S1). Terminal, non-flowering stems of semi-hardened wood approximately 15 cm long were chosen. At this time, differences in flower colour were observed

Table 1 Location and altitude of the Baw-Baw berry (*Wittsteinia vacciniacea*) collections.

Site	Location coordinates	Altitude (m asl)
Mt Baw Baw	−37.839, 146.265	1,459
Mt Cobbler	−37.065, 146.604	1,370
Mt Donna Buang	−37.707, 145.681	1,164
Mt Erica	−37.876, 146.344	1,349
Lake Mountain	−37.504, 145.882	1,039

in plants across the five sites (Fig. 3). Mature fruit was collected from the most extensively populated site, Mt Baw Baw, in April 2023 to provide seed for cultivation.

Propagation

Plant material from the five locations was propagated vegetatively using freshly collected stem cuttings. Seed propagation was tested on freshly collected seed from one site only – Mt Baw Baw. Stem propagation was tested using two different media: Jiffy® Preforma Plugs (Jiffy Products [N.B.] Ltd, Pokemouche, Canada) or propagation trays (a 3:1 vermiculite:perlite mix). As these two methods are commonly used in the Royal Botanic Gardens Victoria (RBGV) nursery for semi-hardwood cuttings, it was decided to incorporate both. The Jiffy® Plugs contained one stem cutting per plug whereas the propagation trays had multiple stem cuttings within the one tray. An additional treatment applied to both media to stimulate root initiation was dipping stem material into +/- Clonex® Rooting Hormone Gel Purple containing 3 g/L of Indole Butyric Acid (Growth Technology Pty Ltd, O'Connor, Western Australia). All treatments included a control in which no hormone gel was applied.

Seed cleaning and quality

Prior to any germination testing, seed from the freshly collected fruits (from Mt Baw Baw) was extracted and cleaned of pulp, and random samples were x-rayed (Faxitron MultiFocus cabinet) to determine the fill rate as a proxy for viability (Merritt *et al*., 2021). To determine seed size, a stereo microscope (Olympus SZX16) with a camera (DP74-CU) was used to measure seed length and seed width at 100 × magnification. Seed was weighed in 100 × five sublots of Mt Baw Baw seed to calculate the average weight.

Germination testing

For the standard testing, 25 seeds were placed on a sterile 90 mm Petri dish with 1 per cent agar or a 90 mm Petri dish with gibberellic acid (GA_3) incorporated in the agar (125 mg/500 ml). All plates were sealed with Parafilm M® and placed into an incubator (Thermoline) with a 20/15 °C temperature setting and 12/12 h photoperiod. Germination was scored weekly.

For the thermogradient design, all seed was collected from the Mt Baw Baw population on 3 April 2023. Seed was 18 days post-harvest when the experiment commenced. Fresh seed was counted (1,960 seeds) and separated into two treatment groups of 980 seeds per treatment. For treatment one (control), 980 seed were soaked in deionised water for 8 h. Treatment two had 980 seed soaked in gibberellic acid, GA_3 (125 mg/500 ml) for 8 h. To ensure the

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Fig. 3 Differences in flower colour of *Wittsteinia vacciniacea* collected from the five sites. From top left: Mt Baw Baw; Mt Erica; Mt Donna Buang; Lake Mountain; and Mt Cobbler. Note the two colours of the Lake Mountain flower are shown here, likely an indication of different stages of maturity, with the more mature flowers exhibiting less red colour (bottom centre). Photos: V. Williamson.

temperature range was covered for both treatments, 196 cells were used, of which 98 cells per treatment were filled in an alternate arrangement (Table S2) using 1 per cent agar in 35 mm sterilised Petri dishes. Ten seed were sown per dish and sealed with Parafilm M® to prevent the agar drying out. Germination was scored weekly, over an eight-week period.

Leaf area

To assess any potential morphological differences within the species from the five sites, we sampled the leaf area of 10 leaves from each of the five locations using a leaf area meter (Li-Cor, Model 3100). Differences in leaf area may be a useful horticultural characteristic.

Statistical analyses

Germination niche

To understand the thermal germination niche, we ran a Generalised Additive Mixed Model (GAMM) with a proportional binomial distribution in 'mgcv' in R (R Core Team & Team, 2022; Wood & Scheipl, 2014).

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The proportion of seeds germinated in each thermogradient grid was used as the response variable. Temperature was included with a smoother to enable non-linear responses. Pre-seed treatment was included as a fixed factor and the interaction between pre-seed treatment and temperature was included. Week since the start of the experiment was also included to assess the time to seed germination. Grid position was included as a random factor to account for repeated sampling of germination through time. We compared this full model with reduced models, including a model without the interaction term, one without the effect of treatment and one without the effect of week. We also considered a null model. We compared the best supported model using Akaike Information Criterion corrected (AICc). Models with an ΔAIC <2 were considered supported.

Environmental niche modelling

We obtained environmental data from CliMond, which provides data formats suitable for correlative environmental niche models (ENMs). For ENM modelling, CliMond provides 35 bioclimatic variables describing their trends, means of quarters and seasonality. We used the quarterly means of the driest period in our models. A grid cell resolution of 10 ′ was selected for both approaches, approximately 20 × 20 km at the equator.

We ran an environmental niche factor analysis (ENFA) in 'adehabitat' in R (Calenge, 2006; R Core Team, 2024) using the 35 variables to identify important variables (O'Donnel & Ignizio, 2012) in determining the distribution of *Wittsteinia vacciniacea*. Variables were ranked by marginality, which describes the difference between the total range of environmental variables and the range occupied by the species within the distributed area. The top 10 variables were then checked for spatial autocorrelation. We generated 10,000 random points and extracted information for each environmental predictor, then performed pairwise Pearson correlation tests on all variables. For highly correlated variables ($r_p > 0.80$), one variable was removed from the pair based on a lower marginality score. By eliminating variables, we could reduce problems caused by multicollinearity and its bias in model predictions. In the MAXENT model, we only enabled hinge features to enforce smooth response curves, as they allowed the model to capture non-linear relationships between environmental variables and species occurrence. Hinge features helped in creating more flexible and realistic response curves that gradually change, which can be particularly useful in modelling species' distributions in complex ecological settings. All other parameters were left at their default. A presence data-only *W. vacciniacea* record from the five sites, including the 51 presence points, was adopted for the MAXENT models. Models were then run 10 times. We used cross-validation for within-model testing (to evaluate predictive performance) and took the average of all replicates for spatial predictions. Each set of predictor variables was examined using the jackknife function of MAXENT and the percentage contribution to the model was determined.

To predict the species distribution, we ran MAXENT (version 3.4.4), which uses the principle of maximum entropy to relate a species' distribution records to environmental variables to estimate the potential distribution of the target organism (MAXENT; Phillips *et al*., 2006). MAXENT was chosen over other modelling algorithms because it has been shown to repeatedly outperform other types of model based on predictive accuracy, and is suitable for presence-only datasets. MAXENT uses information on conditions in the region of interest to compare with conditions at known occurrences. Therefore, the regions must be defined before model construction. We first set the region for the native range to occupied bioclimatic regions from Köppen-Geiger classifications within (or intersecting) the borders of south-eastern Australia, as publications from these areas contributed to all our data points. The Köppen-Geiger classifications followed the rules defined by Kriticos *et al.* (2011) as applied to the 10 ′ resolution CliMond global climatology (sourced from the WORLDCLIM and Climate Research Unit (CRU) datasets).[5] This provides a broad geographical area to account for data availability and bias in occurrence records but omits large geographical areas irrelevant to modelling species distributions.

[5] http://worldclim.org; version 1.4; https://crudata.uea.ac.uk/cru/data/hrg/; version CL2.0

Results

Testing conservation horticultural methods, our results are promising for the introduction of *Wittsteinia vacciniacea* into cultivation through *ex situ* living collections.

Cuttings

Of the 561 plants vegetatively propagated, 341 had root initiation, with an overall success rate of 60.8 per cent, irrespective of treatment. We found treatment differences in the five populations (Table S1), with Lake Mountain showing high root initiation across all treatments. The Mt Erica and Mt Baw Baw populations showed a greater response to the individual Jiffy® Preforma Plugs per cutting than the propagation trays in which cutting material was in rows in a 3:1 vermiculite-perlite mix (Fig. 4).

Germination

Seed size estimates of the Mt Baw Baw population provided baseline trait data:

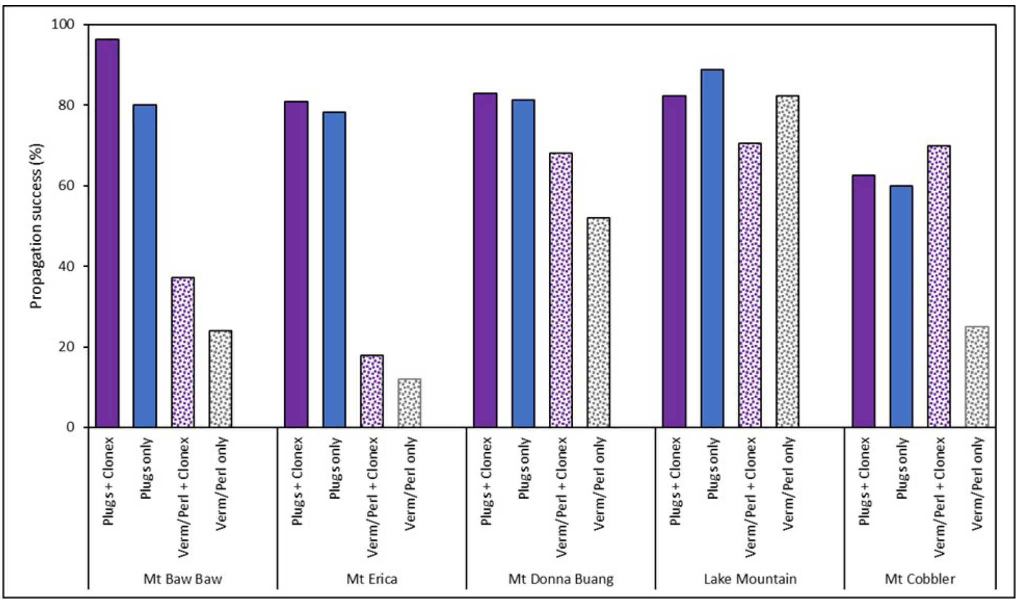

Fig. 4 Propagation results from the five *Wittsteinia vacciniacea* collection sites. Plugs = Jiffy® Preforma Plugs; Clonex = Clonex® Rooting Hormone Gel Purple; Verm/Perl = a 3:1 vermiculite:perlite mix.

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length (μ = 1.9032 mm, SD 0.2137 mm) and width (μ = 1.5783mm, SD 0.1456 mm). Seed quality was high with a 91 per cent fill rate (Fig. S2) and was supported in the 86 per cent germination response under standard testing (data not shown).

Thermogradient

The thermogradient experiment (Table S2) indicated there was strong support for a model with temperature, pre-seed treatment, the interaction between these and the interval between recording germination (week), with the top two models having these variables barely separable (cumulative AIC ώ = 0.896, Table S3). Temperature had a parabolic effect on germination rate, with untreated seed germination occurring between 15 °C and 18 °C. This thermal niche broadened with GA_3 treatment (p = 0.068) to germination between 14 °C and 22 °C (Fig. 5). Per cent germination between 15 °C and 20 °C for both treatments was higher in seed treated with GA_3 (31 per cent, CI: 24–38 per cent) compared to the water pre-treatment (13 per cent, CI: 9–17 per cent). Germination rate increased throughout the study (β = 1.190, 95 per cent CI: 0.93–1.45). Our model explained 92.9 per cent of the deviance within these data.

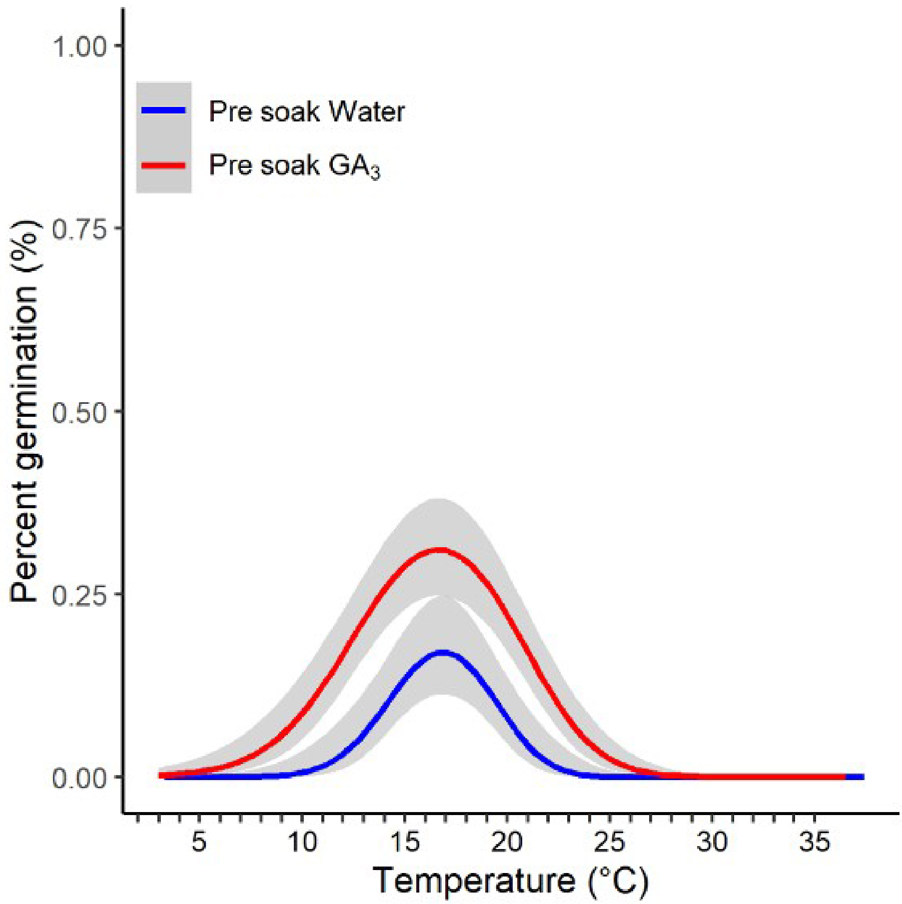

Fig. 5 Thermal germination niche and germination rate of *Wittsteinia vacciniacea* seed within two pre-soak treatments: pre-soak deionised water (blue) and pre-soak gibberellic acid (GA_3) (red).

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Leaf area

There was a significant difference in leaf area between the populations ($F_{4,45} = 6.73$, $p < 0.001$; Fig. 6). Leaves from Mt Donna Buang were significantly larger (Tukey $p < 0.005$) at 5.5 cm^2 than leaves from all other populations which were similar (3.1 cm^2) (Tukey $p > 0.05$).

Environmental niche factor analysis

The ENFA revealed three primary climate variables influencing the distribution of *Wittsteinia vacciniacea*. Temperature of coldest quarter from 0 °C to 10 °C was the best predictor (permutation importance = 61.5, Table S4), with mean diurnal temperature range from 0 °C to 9 °C and isothermality between 30 °C and 42 °C being equally supported (permutation importance = 19.6 and 18.8 respectively; Table S4; Fig. S5). When considering the Köppen-Geiger background, the same three variables were most influential; however, their relative importance changed. Diurnal temperature ranged from 4 °C to 9 °C and was most influential (45.8) closely followed by isothermality between 31 °C and 41 °C (42); mean temperature of the coldest quarter from −1 °C to 6 °C was third most important (Table S4; Fig. S6). Both models had a high predictive accuracy for the species' distribution (AUC = 0.998 and 0.985 respectively). In predicting the species' potential distribution, a distinct region of the Otway Ranges National Park, located in the south-west of Victoria, 162 km from Melbourne, appears suitable. The Otway region was considered a high probability of occurrence in both models, suggesting that detailed surveys for the species in this region are warranted. Several regions in Tasmania also appear suitable to host the species' distribution; however, these areas were deemed less important in the Köppen-Geiger climate models (Fig. 7).

Discussion

As a groundcover plant, *Wittsteinia vacciniacea* lends itself to cultivation due to the ease of vegetative propagation, notably the abundant adventitious roots at regular nodes (Fig. S3). The current experiments supported this with high root initiation, irrespective of treatment. All the propagated

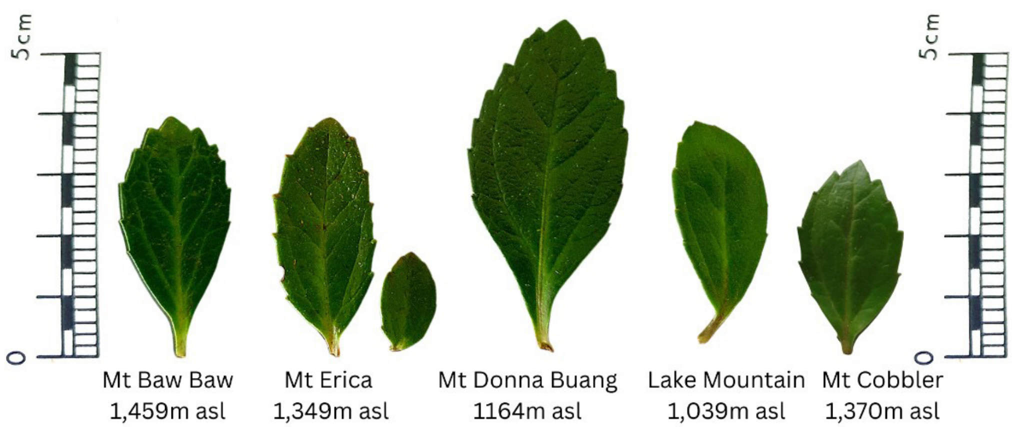

Fig. 6 Example of leaf area differences of *Wittsteinia vacciniacea* from the five collection sites in Victoria. Ten leaf replicates were measured per site. Leaves from Mt Donna Buang were significantly larger (Tukey $p < 0.001$) than leaves from all other populations, which were similar (Tukey $p > 0.05$). Photos: V. Williamson.

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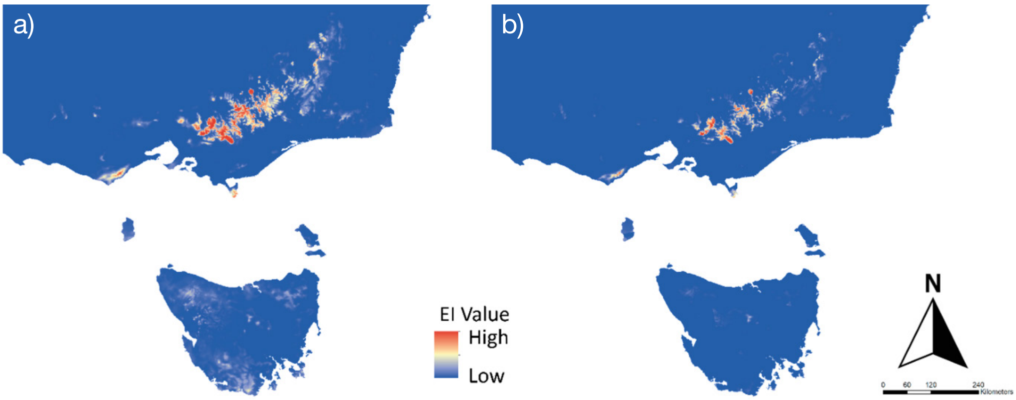

Fig. 7 Environmental niche modelling (ENM) for *Wittsteinia vacciniacea*, using environmental data from CliMond 5 bioclimatic variables. a) is the ENM using all Australia background data; and b) the ENM using only the Köppen-Geiger background. EI stands for Ecoclimatic Index, representing habitat suitability as outputted from the Maxent logistic. The scale ranges from 0 to 1. Red colours indicate a higher suitability for *W. vacciniacea* survival, while blue colours indicate lower suitability.

stem material is now being established as *ex situ* living collections across sites within RBGV.

The thermal niche of untreated fresh seed occurred between 15 °C and 18 °C and broadened under a GA_3 treatment. The germination rates of fresh *Wittsteinia vacciniacea* seed at 86 per cent were greater than the 65 per cent obtained for another Alseuosmiaceae family member, *Alseuosmia quercifolia* A.Cunn. (Merrett *et al.*, 2002), although the present concentration of GA_3 was 10 times higher. The 86 per cent germination success obtained with a 250 mg L^{-1} GA_3 concentration is indicative of an optimal/close to optimal concentration. With this knowledge, *W. vacciniacea* can now confidently be grown using vegetative material to bolster plant numbers across all populations. Additional seed collections could safeguard any potential genetic diversity between populations via storage in an *ex situ* conservation seed bank.

The seed and fruit of *Wittsteinia vacciniacea* are likely dispersed by birds, similar to berries of other Alseuosmiaceae (Merrett *et al*., 2002; Gotty *et al*., 2022), although many *W. vacciniacea* berries were observed to fall directly below the plant, which also occurred in *Alseuosmia quercifolia*, indicating that gravity aids fruit dispersal (Merrett *et al*., 2002). It is unclear whether *W. vacciniacea* is self-pollinated because, although the stamens are introrse, this characteristic also occurs in *A. quercifolia*, which released pollen prior to the stigma becoming receptive (Merrett *et al*., 2002). Seedlings of *W. vacciniacea* were observed growing at the Mt Donna Buang site where seed had clearly been transported downstream and germinated in a wash-out area in which water flow had been slowed by leaf litter (Fig. S4).

Wittsteinia vacciniacea relies on its soil seed bank for reproduction (Commonwealth and Victorian RFA Steering Committee, 1997), so may be at risk from soil disturbance via machinery, especially given its main population is in the ski resort of Mt Baw Baw. In its other locations, *W. vacciniacea* is considered at risk from environmental weed invasion because of its location near potentially weed-prone riparian zones (Commonwealth and Victorian RFA Steering Committee, 1997).

The present experiments revealed that the plants from all populations propagated easily and that there were differences in morphology of the five *Wittsteinia vacciniacea* populations – both beneficial characteristics when bringing this plant into cultivation. The differences in petal colour between populations needs to be explored to determine whether they remain during maturity. The larger leaves in the Mt Donna Buang population can be attributed to the more densely shaded environment provided by an overstory of 30 m high evergreen *Nothofagus cunninghamii* (Hook.) Oerst. Larger leaves in shade are a well-known characteristic, often to support a greater leaf area for optimal photosynthesis (Lichtenthaler *et al*., 1981). There were differences in the leaf margins, with the Lake Mountain population possessing the smallest rosoid teeth (Fig. 6), providing an interesting avenue to pursue in terms of environmental versus genetic differences between the populations. Leaf margins with rosoid teeth are believed to have originated near the base of core ancestral eudicots (Doyle, 2007). The rosoid-toothed leaf margins in Alseuosmiaceae have hydathodes (Dickison, 1989), which means that uncontrolled water loss is likely to occur through such permanently open pores. This could mean that *W. vacciniacea* may be a plant that is limited by its environment and might not survive a drier climate. The areas where *W. vacciniacea* occurs generally have a higher rainfall compared to lowland areas, providing a moist habitat and moisture retention in the deep, humic mountain soils (Morgan, 2004).

Our distribution models predict that there is suitable habitat in the Otway Ranges National Park, where the species has not been recorded as occurring. The Otway Ranges in Victoria is a Gondwana link between the Australian mainland and Tasmania; the same is true of Wilsons Promontory in Victoria's east where a remote population of *Wittsteinia vacciniacea* is also known. It is therefore possible that a population could exist in the Otway Ranges; given the conservation status of the species, investigating its presence there should be a priority. Regardless, this region could represent an ideal location for a trial of *in situ* conservation metrics given the success of *ex situ* horticultural measures considered here (Fig. 8).

Plants that have survived extinction events but which are now marginal are considered by Jablonski (2001) to be a 'dead clade walking'; however, the value to conservation biology of such relict lineages is high due to their representation as 'sole survivors' of unique groups (Vane-Wright *et al*., 1991; Gotty *et al*., 2022). Alseuosmiaceae is believed to be a relict lineage as it contains only four genera and a net dispersal rate half that of the Asterales, of which it is a member, with high extinction rates and no fossil records occurring outside its current range (Gotty *et al*., 2022). The locations of the *Wittsteinia* genus, and indeed other Alseuosmiaceae genera, by being reduced to island refugia, are an indication of long-term decline (Gotty *et al*., 2022). Why *Wittsteinia vacciniacea* has survived in the small, separate mountainous pockets of Victoria is unclear and begs the question of whether it once had a wider range in Australasia/Gondwana. Fluctuating sea levels (Jablonski, 2001) during the Cretaceous period may have resulted in islands of mountainous refugia – referred to as 'islands in the sky' by Mayr & Diamond (1976) for species stranded on mountaintops – where *W. vacciniacea* continues to make its home in Victoria.

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Fig. 8 A *Wittsteinia vacciniacea* plant vegetatively propagated from the experiments now established in the Gondwana Garden, as an *ex situ* living collection, Royal Botanic Gardens Victoria, Cranbourne. Photo: V. Williamson.

Conclusion

The present results support the value of bringing a relict species such as *Wittsteinia vacciniacea* into cultivation and its potential as a horticultural species. The measures undertaken here facilitate greater *ex situ* conservation of the species and could meaningfully be extended to other threatened floral species. A critical next step in the conservation of *W. vacciniacea* is to determine if the differences found here in leaf size and margins are driven primarily by the environment, genetics or a combination of the two. This would be informative at the habitat level, as the areas where *W. vacciniacea* is known to occur provide refuge for the movement, calling sites and breeding micro-habitat of the critically endangered Baw Baw frog and are considered vulnerable to climate change.

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