REPORT: Insect visitors to *Marianthus aquilonaris* and surrounding flora Nov 2-4, 2019

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Background

Marianthus aquilonaris (Fig. 1) was declared as Rare Flora under the Western Australian *Wildlife Conservation Act 1950* in 2002 under the name *Marianthus* sp. Bremer, and is ranked as Critically Endangered (CR) under the International Union for Conservation of Nature (IUCN 2001) criteria B1ab(iii,v)+2ab(iii,v); C2a(ii) due to its extent of occurrence being less than 100 km^2, its area of occupancy being less than 10 km^2, a continuing decline in the area, extent and/or quality of its habitat and number of mature individuals and there being less than 250 mature individuals known at the time of ranking (Appendix A). However, it no longer meets these criteria as more plants have been found, and a recommendation has been proposed to be made by DBCA to the Threatened Species Scientific Committee (TSSC) to change its conservation status to CR B1ab(iii,v)+2ab(iii,v) (Appendix A), but this recommendation has not gone ahead (DEC, 2010). Despite its listing as CR under the Western Australian *Biodiversity Conservation Act 2016,* the species is not currently listed under the *Environment Protection and Biodiversity Conservation Act 1999*. The main threats to the species are mining/exploration, track maintenance and inappropriate fire regimes (DEC, 2010).

Fig. 1. *Marianthus aquilonaris,* showing flower, buds and leaves. Photo: Kit Prendergast Oct 2019

Marianthus aquilonaris is known to occur only in the Bremer Range, which is listed as a Priority 1 Ecological Community (PEC), located approximately 100 km west, south-west of Norseman, Western Australia (Fig. 2, from Botanica Consulting, 2017). The extent of occurrence for this taxon is likely to be less than 0.5 km^2 (DEC, 2010). Subpopulation information is listed in Table 1 in Botanica

Consulting (2017) (refer to Appendix B), however subpopulation updates are forthcoming. Genetic studies suggest limited gene flow between subpopulations (Hopley & Byrne, 2018).

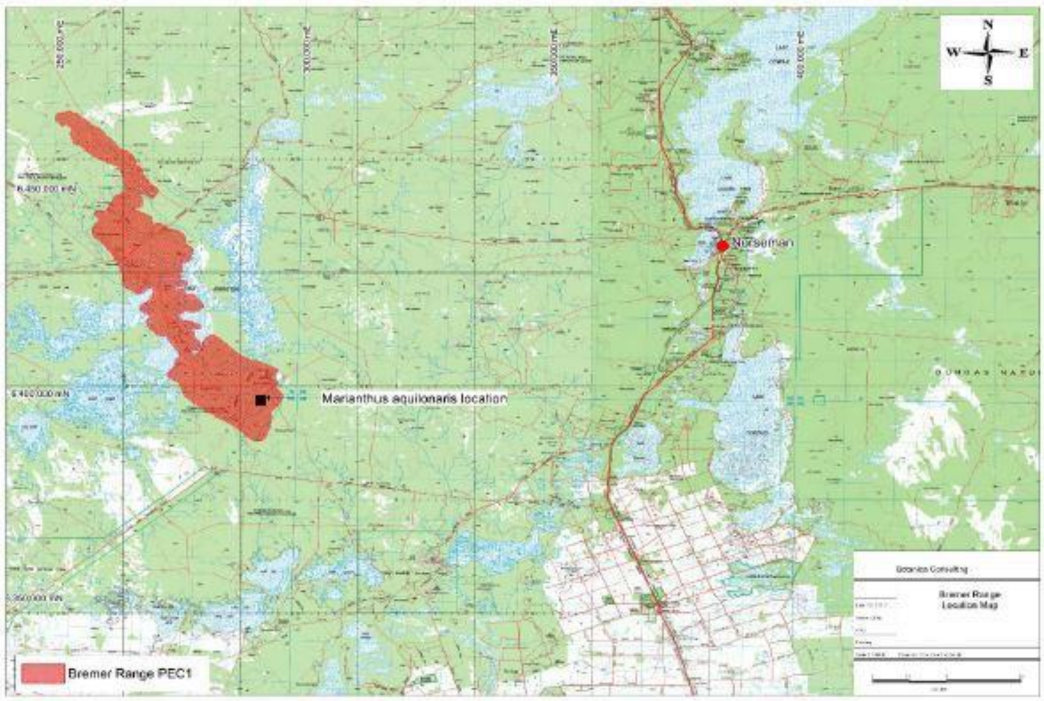

Fig. 2. Map of Bremer Range and *Marianthus aquilonaris* subpopulations.

The aims of this study were to identify the insect visitors to *Marianthus aquilonaris,* and thus establish if it is receiving visits from insects that serve as pollinators, and the identity of these species. Knowledge of the pollinators of this plant can then be used to identify management actions to conserve these floral visitors. Conservation of pollinators is vital if this species is to persist (Prendergast, 2010; Kearns, Inouye & Waser, 1998). This addresses Item 13 in the Environmental Scoping Document (ESD) prepared by Audalia/Preston for assessment of the Project by EPA under the EP Act:

> "Item 13: If potential direct or indirect impacts to *M. aquilonaris* are proposed, identifying potential pollinators for *M. aquilonaris*, including changes to pollinator subpopulations or behaviour, changes to linkages between sub-subpopulations of species pollinated by vectors with short ranges, causing interruptions to gene flow within and between sub-subpopulations." (Preston Consulting Pty Ltd., 2019)

Pollinators are a critical part in the conservation of most angiosperms. The pollination biology of *Marianthus aquilonaris* is unknown, and indeed that of the genus *Marianthus* as a whole is poorly understood, however the small size of the flower and its floral features suggest this genus insect pollinated (Armstrong, 1979). Of all insects, bees tend to be the most effective of pollinators (Willmer et al., 2017). Australia has an estimated 2,000 species of native bees, however a large number of these are undescribed, and the habitat and resource requirements of a large proportion of species are unknown (Batley & Hogendoorn, 2009).

There have been no previous surveys on the insect visitors to *Marianthus aquilonaris.* Other studies by K. Prendergast (Prendergast, in prep.)(Prendergast, 2018a) and records in Houston (2018) on other *Marianthus* species have documented the native bee genera *Amegilla* and *Leioproctus,* as well as the introduced European honeybee *Apis mellifera,* as visitors.

Methodology

The *Marianthus aquilonaris* subpopulations (A – E) at the Audalia Resource Ltd Medcalf Site (Fig. 2, Fig. 3) were surveyed by Kit Prendergast for their insect visitors. In addition, insects were collected in bee bowls and on surrounding flowering vegetation to further investigate potential pollinator species that may also visit *Marianthus aquilonaris.* Collection of insect visitors to *Marianthus aquilonaris,* and bees on surrounding flowering plant species, involved active sampling by K. Prendergast with an entomological sweepnet. In addition, potential insect visitors were also sampled passively using bee bowls.

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Fig. 3. Map of *Marianthus aquilonaris* subsubpopulations.

On Nov 2nd 2019, all *Marianthus aquilonaris* subpopulations were visited between 1400h and 1630h to identify *Marianthus aquilonaris* plants in flower to target during the surveys the following two days. Subpopulation E had no plants in flower and so subsequent insect visitor surveys were conducted at *Marianthus aquilonaris* subpopulations A-D.

Each *Marianthus aquilonaris* subpopulation that had plants blooming was visited for 1-2 hrs to undertake insect collections by Kit Prendergast on Nov 3 and Nov 4 2019 between 0830h and 1530h.

During each survey any *M. aquilonaris* plants in flower were observed for half of the time, and flowering plants surrounding the subpopulation were surveyed for the remainder. Insects were collected with an entomological sweepnet (the most effective method for sampling native bees (Prendergast et al., 2020) and transferred to vials, labelled with the date, subpopulation and plant species and stored in a freezer. All insect taxa visiting *M. aquilonaris* were collected, whereas on plants other than *M. aquilonaris* only bees were collected.

In addition to the active collecting, insects were collected passively using bee bowls (also known as pan traps), which comprised 12 oz. plastic bowls filled with water and a few drops of detergent which acted as a surfactant, lowering the surface tension of the water to prevent insects caught in the bowls from flying out. At each subpopulation in the morning one fluoro yellow and one fluoro blue bowl (colours attractive to bees (Prendergast et al., 2020)) were placed near *Marianthus aquilonaris* plants with the most flowers, and were checked in the afternoon to collect any bees that had been captured in the bowls (Fig. 4, see also Appendix C). The bowls were also left overnight on Nov 3 2019 and checked for specimens the following morning to account for the potential to collect nocturnal pollinators or taxa that continued to forage after active surveys had concluded for the day.

Fig. 4. Yellow (a) and blue (b) bee bowls. Note *Lasioglossum (Chilalictus)* bees (a, b, c) and *Amegilla chlorocyanea* bee (b, d). Photos: Kit Prendergast

Insects were later thawed, pinned, labelled, and identified to the lowest taxonomic level possible by K. Prendergast using keys, published descriptions, and with reference to the WA Museum entomological collection.

Results and Discussion

A number of potential pollinating insect species were collected visiting *Marianthus aquilonaris*, and in addition, a high diversity of native bee species were recorded in the area. However more work on the biology and ecology of the species visiting the plants is required, and further pollinator surveys are required due to the current surveys being conducted outside of the peak bloom period of *M. aquilonaris.*

During the surveys, a total of 317 native bees belonging to 47 species were collected (Appendix D, Table D1). However, only a small fraction of these native bees (15 individuals belonging to six species) were visitors to *Marianthus aquilonaris* (Table 1). The vast majority of individuals and species were collected on *Eucalyptus livida*, which hosted a prolific number of native bees as well as other insects (Appendix D, Table D1).

Table 1. Insect visitors collected on *Marianthus aquilonaris* flowers.

Species	Total no. recorded visiting *Marianthus aquilonaris*	Sex	Number of individuals	*Marianthus aquilonaris* subpopulation	Date of collection
Bees					
Lasioglossum (Chilalictus) florale	2	M	1	D	3/11/2019
		F	1	D	3/11/2019
Xanthesma sp	1	M	1	A	4/11/2019
Lasioglossum (Chilalictus) castor	1	F	1	A	4/11/2019
Megachile 66 "shelf clypeus"	1	F	1	A	4/11/2019
Megachile maculosipes	1	M	1	A	4/11/2019
Megachile 65 "prongs"	1	F	1	C	4/11/2019
Flies					
Syrphidae Sp.1	1			A	4/11/2019
Bombyliidae *Geron* sp.1	2			A	4/11/2019

There were very few *Marianthus aquilonaris* plants in flower – they had largely ceased flowering. Of the 5,712 live plants (DBCA Live Total Count (2015), from Botanica Consulting, 2017), less than fifty were in flower, and of those that were, the number of flowers on the plants ranged from 1 – 10, typically four (see Appendix C, Figs C1-C4). Peak flowering occurred late Sept/early Oct (DEC 2010) (initial proposed survey date was planned for this time period but was delayed). This would have affected the outcome of this study, in that due to the survey period falling outside of peak bloom, it is likely that the results here are a conservative picture of the insect visitors to *M. aquilonaris*, and when in peak bloom a greater number of individuals, and potentially other species, would be collected.

Conditions were quite dry (Bureau of Meteorology, 2020), and there were few other plant species in flower. The plants species besides *Marianthus aquilonaris* in bloom were: *Eremophila caperata* (common but only a few flowers per plant); Solanaceae sp. (only four plants, but with numerous flowers, on the track away from the *M. aquilonaris*); *Halgania lavandulacea* (common but only a few flowers per plant); *Eucalyptus livida* (a number of trees near *Marianthus aquilonaris* with 5-50 blossoms, but still not flowering profusely across the landscape); *Asteridea athrixioides* (one plant, but many flowers, near subpopulation D); *Scaevola spinescens* (fairly common, approx. 20 flowers per plant); *Waitzia fitzgibbonii* (relatively abundant at subpopulation D); native Hibiscus (*Alyogyne ?hakeifolia*) (some distance from subpopulation A) (see Appendix E, Table E1).

Visitors to Marianthus aquilonaris

Native bee taxa visiting *Marianthus aquilonaris* included a tiny species of native bee (Colletidae: Euryglossinae, *Xanthesma* undescribed sp. 60, male), two *Lasioglossum* species (*Lasioglossum (Chilalictus) castor*, female, and *Lasioglossum (Chilalictus) florale*, male and female) (Appendix F, Fig. F1), two *Megachile* species (*Megachile maculosipes*, male and an undescribed species, *Megachile* 66 F "shelf clypeus", female), and one undescribed *Megachile* (*Megachile* 65 F "prongs", female) (Appendix F, Fig. F2d) (Table 1, see also Appendix D, Table D1).

In addition, three flies (Diptera) were observed visiting *Marianthus aquilonaris*: two tiny flies (*Geron* sp., Bombyliidae) were collected on the flowers in the afternoon on 3 Nov 2019 at Subpopulation A and a hoverfly (Syrphidae) at Subpopulation D on 4 Nov 2019 (Table 1). Whether these fly taxa serve as pollinators is unknown, as although flies can be pollinators (Inouye, Larson, Ssymank, & Kevan, 2015), they can also be nectar thieves and are generally less effective at pollinating than bees (Willmer, Cunnold, & Ballantyne, 2017).

With three of the native bee species collected foraging on *Marianthus aquilonaris* being undescribed, and potentially even new to science, their range and potential conservation status is entirely unknown. A similar situation exists for *Megachile maculosipes* is not officially recognised, having been named and published in a thesis (King, 1986). Further studies and surveys to determine the range of these species, identify their habitat requirements, and food and nesting resource requirements are therefore required.

For the three megeachilid species (genus *Megachile*) however it is likely that, like most species in this genus, that they rely on old, large trees that contain small cavities created by wood-boring beetles for nesting substrates (Morato & Martins, 2006; Sydenham et al., 2016). Therefore any activity that removes trees or impacts the beetles they rely on for cavities represents a threat to these bees, which are generally the most effective of pollinators due to the scopae being located on the underside of the abdomen.

The sole euryglossine bee that was collected on *M. aquilonaris* was an undescribed *Xanthesma* species; consequently whilst this species specific range and habitat requirements are unknown, this genus is known to nest in soil (Houston, 1969).

The two halictids collected - *Lasioglossum (Chilalictus) florale* and *Lasioglossum (Chilalictus) castor* - are both described and published information on their biology exists. Both species have a wide range: *L. castor* occurs throughout southwest Western Australia (Walker, 1995), and can be locally abundant and is a common component of bee assemblages (K. Prendergast, unpublished data). The

geographic range of *Lasioglossum (Chilalictus) florale* encompasses most of mid-west, south-west and southern Australia, and it is known to be locally abundant in some locations (Walker, 1995). Interestingly, both species have yet to be collected on a plant species within the family Pittosporaceae, however they are both polylectic species, visiting a high taxonomic diversity of plant species (Walker, 1995; K. Prendergast, unpublished data). *Lasioglossum (Chilalictus)* species nest in the ground (Walker, 1995).
For the ground-nesting bee species, any activities that disturb suitable nesting substrate (e.g. earthworks, road construction, mining) would harm these pollinators.

Taxa caught in bee bowls

165 insects were captured in the bee bowls (68 in the blue bowls and 97 in the yellow bowls); of these 127 were native bees (44 captured in the blue bowls and 83 in the yellow bowls) (Table 2). The higher catch rates of native bees in the blue bowls than yellow are consistent with previous studies by K. Prendergast (Prendergast et al., 2020). Bees collected passively in the bee bowls next to *Marianthus aquilonaris* included species that are effective pollinators (Michener, 2007), including the large, mobile *Amegilla* (Houston, 2018). Morever *Amegilla* has been observed to visit another *Marianthus* species (*M. bicolor*) (K. Prendergast, in prep.). Whilst this establishes that native bee taxa occur in the close vicinity of *M. aquilonaris,* the lack of observations of these taxa visiting the plants combined with the genetic data (Botanica Consulting, pers. comm., 2019) suggest that they seldom if at all visit the target plant species, however studies when *M. aquilonaris* is in peak flower would be required to establish this.
The numbers of bees collected in bee bowls next to *M. aquilonaris* far exceeded the number actually foraging on the plants. This highlights a pitfall of bee bowls in that they cannot demonstrate bees actually foraging on the plants (Prendergast et al., 2020). Metabarcoding studies of the bees collected however would reveal if pollen in the gut contents of bees in the bee bowls contained sequences matching *M. aquilonaris*. Although bee bowls collected more bees than sweepnetting from *M. aquilonaris*, overall the number and diversity collected by sweepnetting overall far exceeded that collected by sweepnetting, in line with previous studies by K. Prendergast in the urbanised region of southwest Western Australia (Prendergast et al., 2020).

Table 2. Bee and fly taxa collected in bee bowls near *Marianthus aquilonaris*

Bee Bowl colour	Species	Date collected	*Marianthus aquilonaris* Sub-population	Sex	No. collected	Total No.
Blue	*Amegilla (Notomegilla) chlorocyanea*	3/11/2019	A	F	1	3
		4/11/2019	A	F	1	
		4/11/2019	B	F	1	
	Megachile 65 "prongs"	3/11/2019	B	F	1	5
		4/11/2019	B	F	1	
		4/11/2019	D	F	3	
	Megachile carnaua	4/11/2019	D	F	1	1
	Lasioglossum (Chilalictus) castor	3/11/2019	C	F	1	20

		4/11/2019	A	F	1	
		4/11/2019	B	F	5	
		4/11/2019	C	F	8	
		4/11/2019	C	M	1	
		4/11/2019	D	F	4	
	Lasioglossum (Chilalictus) erythrurum spp-group	4/11/2019	C	F	3	4
		4/11/2019	D	F	1	
	Lasioglossum (Chilalictus) cf. sexsetum	4/11/2019	C	F	1	2
		4/11/2019	D	F	1	
	Lasioglossum (Chilalictus) cf. victoriellum	4/11/2019	D	F	1	1
	Lasioglossum (Chilalictus) cf. greavesi	4/11/2019	D	F	1	1
	Lipotriches (Austronomia) hippophila	4/11/2019	D	F	1	1
	Lipotriches (Austronomia) flavovridis spp-group	4/11/2019	D	F	1	1
	Diptera: Syrphidae sp.1	4/11/2019	D		1	1
Yellow	*Amegilla (Notomegilla) chlorocyanea*	3/11/2019	A	F	1	1
	Homalictus (Homalictus) cf. urbanus	3/11/2019	A	F	1	2
		3/11/2019	A	M	1	
	Lasioglossum (Chilalictus) castor	3/11/2019	A	F	1	40
		4/11/2019	A	F	13	
		4/11/2019	B	F	2	
		4/11/2019	C	F	10	
		4/11/2019	D	F	14	
	Lasioglossum (Chilalictus) cf. occiduum	4/11/2019	C	F	1	1
	Lasioglossum (Chilalictus) cf. instabilis	4/11/2019	C	F	1	5
		4/11/2019	A	F	4	
	Euhesma (Euhesma) balladonia/walkeri	4/11/2019	A	F	1	2
		4/11/2019	B	F	1	
	Euhesma (Euhesma) inconspicua	4/11/2019	B	F	1	1

Lasioglossum (Chilalictus) cf. ptyon	4/11/2019	A	F	2	2
Lasioglossum (Chilalictus) cf. sexsetum	4/11/2019	A	F	2	2
Megachile 65 "prongs"	4/11/2019	B	F	2	9
	4/11/2019	C	F	1	
	4/11/2019	D	F	6	
Megachile clypeata	4/11/2019	B	F	1	1
Megachile 66 "shelf clypeus"	4/11/2019	D	F	1	1
Megachile 68	4/11/2019	D	M	1	1
Diptera: Syrphidae sp.1	4/11/2019	A		1	5
	4/11/2019	D		4	

Implications for Marianthus aquilonaris pollination

The relative paucity of insect visitors to *Marianthus aquilonaris* observed during these surveys cannot be taken as conclusive evidence that few insects visit this species. Due to visiting well after peak flowering, the few scattered flowers did not represent an attractive foraging resource for bees, which are known to target larger, clumped patches of flowers (Cresswell & Osborne, 2004; Sih & Baltus, 1987). Nevertheless, despite being well after peak bloom, over the two days of surveys, K. Prendergast collected a total of 15 insect visitors to *M. aquilonaris*, of which 11 were native bees belonging to a number of genera. It is evident therefore that *M. aquilonaris* is not experiencing pollinator deficits, and it is highly likely that a far greater abundance and diversity of pollinators would visit the plants during peak bloom.

Megachile and *Lasioglossum* are both effective pollinators of many taxa (Michener, 2007). *Megachile* in particular are highly effective as pollinators, as the scopae are located on the underside of the abdomen, in a prime location for transferring pollen to the stigma of flowers (Michener, 2007). The Euryglossinae are unlikely to be effective pollinators (in terms of cross-pollination), due to their small size (with larger bees being better pollinators (Willmer & Finlayson, 2014)) and how they swallow pollen and are relatively hairless (Michener, 2007). Nevertheless, euryglossines are known to be pollinators of native flora, and have evolved many specialised, co-evolutionary specialised relationships (e.g. Exley, 1998; Houston, 1983). Euryglossinae are a key part of Australia’s bee biodiversity, being the most species-rich of all subfamilies, and are largely endemic to Australia (Houston, 2018). New species are continually being discovered and described (Hogendoorn, Stevens, & Leijs, 2015).

There was abundant seedset during the current surveys, evidenced by many seed pods on the plants. This suggest that pollination is occurring, but based on the genetic data, there is little pollen exchange between plants of different sub-populations (Hopley & Byrne 2018a; Hopley & Byrne, 2019b). This suggests that either a) the pollinators of this plant have low vagility and/or small flight ranges, and/or generally forage on flowers in the same plant or between adjacent plants; or b) seed set is mostly a result of selfing and potentially wind pollination resulting in only local pollen transfer. Therefore, whilst the genetic data do suggest that visitation is rare, or only by insects with low vagility, further pollination studies to determine the contribution of insects to seed production are required, which would involve:

a) surveys of insect visitors to the plants during peak bloom;
b) investigation of pollen loads on insect visitors;
c) pollination studies involving bagging some flowers on multiple plants (thereby excluding insect visitors) and comparing seed set between bagged and open (control) flowers;
d) hand pollination trials to determine whether pollen transferred from stamens of the same flower, same plant, plants in the same subpopulation, and plants in other subpopulations, all result in the production of seedpods.

The small body size of some of the insects observed foraging on *Marianthus aquilonaris* is in line with the genetic data (Hopley & Byrne 2018a; Hopley & Byrne, 2019b): flight distance is directly related to body size (Gathmann & Tscharntke, 2002; Greenleaf, Williams, Winfree, & Kremen, 2007). With bees being central place forages (Westrich, 1996), nesting sites and foraging resources must be within the flight range of the species. As *M. aquilonaris* subpopulations are separated by >500 m, it may be that the native bees are rarely flying between subpopulations, thereby explaining the limited pollen exchange.

Few young *M. aquilonaris* plants were observed during the surveys (K. Prendergast, pers. obs., 2019). These observations, together with genetic data showing little pollen exchange between subpopulations, and no to very poor germination (Botanica Consulting, 2017) suggest that *M. aquilonaris* is suffering from inbreeding depression (Harmon & Braude, 2010). The current surveys established that *M. aquilonaris* is pollinated by bees, including those that are effective pollinators. It appears therefore that the lack of pollen exchange between subpopulations may be due to the subpopulations being fragmented and exceeding the flight range of the bees (Aizen & Feinsinger, 1994; Brosi, Daily, Shih, Oviedo, & Durán, 2008; Donaldson, Nänni, Zachariades, & Kemper, 2002; González-Varo, Arroyo, & Aparicio, 2009; Hunter, 2002; Murren, 2002; Newman, Ladd, Brundrett, & Dixon, 2013).

The current surveys did establish that an incredibly abundant and diverse native bee assemblage are present in the vicinity of *Marianthus aquilonaris*, largely foraging on *Eucalyptus livida* (Appendix F, Fig. F2, Appendix G). With such a high diversity and abundance of native bees, this rules out the hypothesis that the low genetic variation between subpopulations (Hopley & Byrne 2018a; Hopley & Byrne, 2019b) is due to an absence of bees – the primary and most effective pollinators for most angiosperms (Willmer et al., 2017).

Many of the bee species were collected on *Eucalyptus livida*, and some of these taxa (Hylaeinae, Euryglossinae) are known to specialise on Myrtaceae. However, specialisation in bees is considered in terms of pollen resources, not nectar, and therefore these bees may forage on other taxa, including *Marianthus aquilonaris*, for nectar. This would be likely when the *M. aquilonaris* was in peak bloom, representing a readily-available nectar resource.

Very few European honeybees (*Apis mellifera*) were observed, and none were observed foraging on any of the flora, with all observations occurring of honeybees around tiny depressions containing water. The relative paucity of honeybees may be due to the scarcity of water, and the large distances from domesticated hives, given that domestic hives represent both a source from which domesticated honeybee foragers can come from, as well as a source from which feral honeybee colonies can establish from when a colony swarms. The relative scarcity of honeybees may in fact play a role in the abundance and diversity of native bee taxa, given there is some evidence that this introduced species may be having detrimental impacts on wild indigenous bees, including Australian bees (Prendergast, 2018b; Prendergast et al., in prep).

Conclusion

These surveys have established that the region supports a rich diversity of native bees, and thus is of high conservation value for native bee biodiversity. Of sites previously surveyed by K. Prendergast across Western Australia, this level of native bee biodiversity has yet to be recorded in a given season at a single site (K. Prendergast, unpublished data).

Despite the limitations of surveys being conducted outside of peak flowering time of *Marianthus aquilonaris,* the surveys fulfilled the aims of this project with respect to the EOD:

> "Item 13: If potential direct or indirect impacts to *M. aquilonaris* are proposed, identifying potential pollinators for *M. aquilonaris*, including changes to pollinator subpopulations or behaviour, changes to linkages between sub-subpopulations of species pollinated by vectors with short ranges, causing interruptions to gene flow within and between sub-subpopulations" (Preston Consulting Pty Ltd., 2019)

Six species of native bees were collected on *Marianthus aquilonaris*: an undescribed *Xanthesma* sp. 60 (family Colletidae), *Lasioglossum (Chilalictus) castor* (family Halictidae), *Lasioglossum (Chilalictus) florale* (family Halictidae), *Megachile maculosipes* (family Megachilidae), undescribed *Megachile* 66 F "shelf clypeus" (family Megachilidae), undescribed *Megachile* 65 F "prongs" (family Megachilidae). In addition, two fly species, in the family Syrphidae and Bombyliidae, were also recorded.

Only two of the native bee species have published information about their biology, and hence further studies on the remaining species is warranted, including identifying how restricted in distribution the undescribed species collected at this locality are. Knowledge on the biology of these species based on their generic classification however indicates that undisturbed soil and mature trees are required to support their nesting and therefore reproductive activities. The native bee taxa were small to medium-sized, and therefore have limited flight ranges (Zurbuchen et al., 2010). As bees are central-place foragers, their foraging and nesting resources must be within flight range (Michener, 2007). With genetic data on *Marianthus aquilonaris* suggesting limited pollen exchange between the sub-populations, it appears that the sub-populations are isolated from the perspective of these pollen vectors. Any activity that may further isolate the sub-populations through destruction of nesting resources, or a shrinking of the area of occupancy of the *Marianthus aquilonaris* plants, may further impede pollen flow between the sub-populations.

The limitations in few *Marianthus aquilonaris* plants being in flower means that the full suite of insect visitors could not be established, however K. Prendergast was able to reveal that a range of insect taxa, including species of native bees that are effective pollinators, visited this species.

These surveys also clearly demonstrated the importance of *Eucalyptus livida* as a foraging resource for supporting native bee biodiversity in the vicinity of *Marianthus aquilonaris.* Representing a rich supply of nectar and pollen visited by a diverse taxa, these trees represent important foraging resources for native bees, including the pollinators of *Marianthus aquilonaris.*

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Appendix A: International Union for Conservation of Nature (IUCN) Threatened Species categories

Species are assigned the following categories: Extinct, EX Near Threatened, NT Extinct in the Wild, EW Least Concern, LC Critically Endangered, CR Data Deficient, DD Endangered, EN Not Evaluated, NE Vulnerable, VU. For the criteria for Critically Endangered, Endangered and Vulnerable there is a hierarchical alphanumeric numbering system of criteria and subcriteria. These criteria and subcriteria form an integral part of the Red List assessment and all those that result in the assignment of a threatened category must be specified after the category.

SUMMARY OF THE FIVE CRITERIA (A-E) USED TO EVALUATE IF A TAXON BELONGS IN AN IUCN RED LIST THREATENED CATEGORY (CRITICALLY ENDANGERED, ENDANGERED OR VULNERABLE).[1]

A. Population size reduction. Population reduction (measured over the longer of 10 years or 3 generations) based on any of A1 to A4

	Critically Endangered	Endangered	Vulnerable
A1	≥ 90%	≥ 70%	≥ 50%
A2, A3 & A4	≥ 80%	≥ 50%	≥ 30%

A1 Population reduction observed, estimated, inferred, or suspected in the past where the causes of the reduction are clearly reversible AND understood AND have ceased.

A2 Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

A3 Population reduction projected, inferred or suspected to be met in the future (up to a maximum of 100 years) *[(a) cannot be used for A3].*

A4 An observed, estimated, inferred, projected or suspected population reduction where the time period must include both the past and the future (up to a max. of 100 years in future), and where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

based on any of the following:

(a) direct observation *[except A3]*

(b) an index of abundance appropriate to the taxon

(c) a decline in area of occupancy (AOO), extent of occurrence (EOO) and/or habitat quality

(d) actual or potential levels of exploitation

(e) effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

B. Geographic range in the form of either B1 (extent of occurrence) AND/OR B2 (area of occupancy)

	Critically Endangered	Endangered	Vulnerable
B1. Extent of occurrence (EOO)	< 100 km^2	< 5,000 km^2	< 20,000 km^2
B2. Area of occupancy (AOO)	< 10 km^2	< 500 km^2	< 2,000 km^2
AND at least 2 of the following 3 conditions:			
(a) Severely fragmented **OR** Number of locations	= 1	≤ 5	≤ 10

(b) Continuing decline observed, estimated, inferred or projected in any of: **(i)** extent of occurrence; **(ii)** area of occupancy; **(iii)** area, extent and/or quality of habitat; **(iv)** number of locations or subpopulations; **(v)** number of mature individuals

(c) Extreme fluctuations in any of: **(i)** extent of occurrence; **(ii)** area of occupancy; **(iii)** number of locations or subpopulations; **(iv)** number of mature individuals

C. Small population size and decline

	Critically Endangered	Endangered	Vulnerable
Number of mature individuals	< 250	< 2,500	< 10,000
AND at least one of C1 or C2			
C1. An observed, estimated or projected continuing decline of at least (up to a max. of 100 years in future):	25% in 3 years or 1 generation (whichever is longer)	20% in 5 years or 2 generations (whichever is longer)	10% in 10 years or 3 generations (whichever is longer)
C2. An observed, estimated, projected or inferred continuing decline AND at least 1 of the following 3 conditions:			
(a) **(i)** Number of mature individuals in each subpopulation	≤ 50	≤ 250	≤ 1,000
(ii) % of mature individuals in one subpopulation =	90–100%	95–100%	100%
(b) Extreme fluctuations in the number of mature individuals			

D. Very small or restricted population

	Critically Endangered	Endangered	Vulnerable
D. Number of mature individuals	< 50	< 250	**D1.** < 1,000
D2. *Only applies to the VU category* Restricted area of occupancy or number of locations with a plausible future threat that could drive the taxon to CR or EX in a very short time.	-	-	**D2.** typically: AOO < 20 km^2 or number of locations ≤ 5

E. Quantitative Analysis

	Critically Endangered	Endangered	Vulnerable
Indicating the probability of extinction in the wild to be:	≥ 50% in 10 years or 3 generations, whichever is longer (100 years max.)	≥ 20% in 20 years or 5 generations, whichever is longer (100 years max.)	≥ 10% in 100 years

1 Use of this summary sheet requires full understanding of the *IUCN Red List Categories and Criteria* and *Guidelines for Using the IUCN Red List Categories and Criteria*. Please refer to both documents for explanations of terms and concepts used here.

Source: IUCN. (2012). IUCN Red List Categories and Criteria: Version 3.1. Second edition.Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp. Available to download: https://www.iucnredlist.org/resources/summary-sheet

Appendix B: Summary of *Marianthus aquilonaris* sub-populations

Table 1: Summary of *Marianthus aquilonaris* sub-populations

Population No.	DBCA Live Total Count (2011)[1]	Botanica Live Total Count (2013/2014)[2]	DBCA Live Total Count (2015)[3]	Area Occupied (m^2)[4]	Population Condition[4]
1a	9820	260**	2259	25,288	Moderate
1b	787	138**	247	5,645.5	Moderate
1c	7091	1142**	3205	16,719	Healthy
1d	N/A*	2090	NOT COUNTED	25,400	Healthy
1e	N/A*	1029	NOT COUNTED	2,200	Healthy
1f	N/A*		1	11	Healthy
TOTAL	**17,698**	**4,659**	**5,712**	**75,263.5**	

[1] Population monitoring conducted by DBCA in October 2011.
[2] simple plant count conducted by Botanica 2013-2014 (yellow shading=2013; orange shading=2014)
[3] simple plant count conducted by DBCA 29th September 2015 (listed on the TPFL database).
[4] area occupied/ population condition as listed on DBCA TPFL database based on assessments conducted by Botanica and DBCA.
*N/A-Sub-populations were not identified during the 2011 count conducted by DBCA
**Simple count of mature plants only not full record of all plants present.

Source: Botanica Consulting (2017). Memorandum: *Marianthus aquilonaris* to Geoffrey Hann (Audalia Resources Limited). Botanica Consulting, Western Australia, p.3.

Appendix C: *Marianthus aquilonaris* subpopulations

Fig. C1: Subpopulation A

Fig. C2: Subpopulation B

Fig. C3: Subpopulation C

Fig. C4: Subpopulation D

Fig. C5: Landscape in which the *Marianthus aquilonaris* subpopulations occur; note *M. aquilonaris* plants in the foreground, lacking flowers.

Appendix D: Specimens collected

Table D1. Total number of potential pollinator taxa collected during the surveys of *Marianthus aquilonaris* by K.S. Prendergast, Nov 2 – Nov 4, 2019.

Date	Population (Way Point)	Lat/Long GDA94	Host	Family	Species Code	Species	no. female	no. male	no. per bee species
2.Nov. 2019	A (35, 36)	[redacted]	*Eucalyptus livida*	Colletidae	Euryglossinae 63 M	*Callohesma lucida*		1	1
				Halictidae	Lasioglossum 27 F	*Lasioglossum (Chilalictus) mediopolitum*	1	1	2
	C (33)	[redacted]	*Eucalyptus livida*	Colletidae	Euryglossinae 61 F	*Xanthesma (Argohesma) nukarnensis*	1		1
	D (32, 41)	[redacted]	*Eucalyptus livida*	Colletidae	Hylaeus 13 F	*Hylaeus (Prosopisteron) chlorosoma*	1	1	2
			Marianthus aquilonaris	**Halictidae**	**Lasioglossum 35 F**	***Lasioglossum (Chilalictus) florale***	**1**	**1**	**2**
3.Nov. 2019	A (35, 36)	[redacted]	BlueBeeBowl	Apidae	Amegilla 1 F	*Amegilla (Notomegilla) chlorocyanea*	1		1
			Eucalyptus livida	Colletidae	Euryglossinae 61 F	*Xanthesma (Argohesma) nukarnensis*	4	21	25
				Colletidae	Hylaeus 13 F	*Hylaeus (Prosopisteron) chlorosoma*	2	3	5
				Colletidae	Hylaeus 16 F	*Hylaeus (Gnathoprosopis) amiculus*	4	1	5
				Colletidae	Hylaeus 62 F	*Hylaeus (Rhodohylaeus) lateralis*	2	3	5
				Colletidae	Euryglossinae 4F	*Euryglossula sp. 4 F*	2		2
				Colletidae	Euryglossinae 5 F	*Hyphesma astronomicans*	2		2
				Colletidae	Hylaeus 6 F	*Hylaeus (Rhodohylaeus) proximus*	2		2
				Colletidae	Hylaeus 13 F	*Hylaeus (Prosopisteron) chlorosoma*	2		2
				Colletidae	Euryglossinae 17 F	*Euryglossina (Turnerella) argocephala*	1		1
				Colletidae	Euryglossinae 58 M	*Brachyhesma (Brachyhesma) wyndhami*		1	1
				Colletidae	Euryglossinae 59 F	*Pachyprosopis (Parapachyprosopis) eucyrta*	1		1
				Colletidae	Euryglossinae 64 M	*Callohesma sinapipes*		1	1

				Colletidae	Hylaeus 64 M	*Hylaeus (Euprosopis) honestus*		1	1
				Colletidae	Hylaeus 65 M	*Hylaeus (Rhodohylaeus) "crassigenatus*		1	1
				Colletidae	Euryglossinae 53 M	*Pachyprosopis (Pachyprosopis) haematostoma*		1	1
				Colletidae	Euryglossinae 54 F	*Euhesma (Euhesma) cf. nitidifrons*	1		1
				Colletidae	Leioproctus 9 M	*Leioproctus (Leioproctus) clarki*		1	1
				Halictidae	Lasioglossum 32? M	*Lasioglossum (Chilalictus) cf. occiduum*		2	2
				Halictidae	Lasioglossum 28 F	*Lasioglossum (Chilalictus) cf. greavesi*	2		2
				Halictidae	Homalictus 6 F	*Homalictus 6*	1		1
				Halictidae	Lasioglossum 30 M	*Lasioglossum (Chilalictus) erythrurum spp-group*		1	1
				Halictidae	Lasioglossum 37 M	*Lasioglossum (Chilalictus) cf. ebeneum*		1	1
				Halictidae	Lipotriches 7 M	*Lipotriches (Austronomia) hippophila*		1	1
				Halictidae	Lasioglossum 27 M	*Lasioglossum (Chilalictus) mediopolitum*		1	1
			YellowBeeBowl	Apidae	Amegilla 1 F	*Amegilla (Notomegilla) chlorocyanea*	1		1
				Halictidae	Homalictus 7 F	*Homalictus (Homalictus) cf. urbanus*	1	1	2
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	1		1
	B (34)	[redacted]	BlueBeeBowl	Megachilidae	Megachile 65 F	*Megachile 65 "prongs"*	4		4
			Eucalyptus livida	Colletidae	Euryglossinae 61 F	*Xanthesma (Argohesma) nukarnensis*	2	16	18
				Colletidae	Hylaeus 62 F	*Hylaeus (Rhodohylaeus) lateralis*	4	7	11
				Colletidae	Hylaeus 16 F	*Hylaeus (Gnathoprosopis) amiculus*	3	2	5
				Colletidae	Hylaeus 6 F	*Hylaeus (Rhodohylaeus) proximus*	1	3	4
				Colletidae	Hylaeus 65 M	*Hylaeus (Rhodohylaeus) "crassigenatus*		4	4
				Colletidae	Hylaeus 30 M	*Hylaeus (Euprosopis) elegans*		2	2
				Colletidae	Euryglossinae 53 M	*Pachyprosopis (Pachyprosopis) haematostoma*		1	1
				Colletidae	Euryglossinae 58 F	*Brachyhesma (Brachyhesma) wyndhami*	1		1
				Colletidae	Hylaeus 13 M	*Hylaeus (Prosopisteron) chlorosoma*		1	1
				Colletidae	Leioproctus 9 M	*Leioproctus (Leioproctus) clarki*		1	1
				Halictidae	Lasioglossum 30 F	*Lasioglossum (Chilalictus) erythrurum spp-group*	1		1

	C (33)	[redacted]	BlueBeeBowl	Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	1		1
	D (32, 41)	[redacted]	*Eucalyptus livida*	Halictidae	Lasioglossum 27 F	*Lasioglossum (Chilalictus) mediopolitum*	2		2
				Halictidae	Homalictus 6 F	*Homalictus 6*	1		1
4.Nov. 2019	A (35, 36)	[redacted]	BlueBeeBowl_am	Apidae	Amegilla 1 F	*Amegilla (Notomegilla) chlorocyanea*	1		1
			BlueBeeBowl_pm	Coleoptera	Buprestidae sp.1	*Buprestidae sp.1*	1		1
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	2		2
			Eucalyptus livida	Apidae	Exoneura 9 F	*Exoneura 9*	1		1
				Colletidae	Euryglossinae 61 F	*Xanthesma (Argohesma) nukarnensis*	4	23	27
				Colletidae	Hylaeus 16 F	*Hylaeus (Gnathoprosopis) amiculus*	10	1	11
				Colletidae	Euryglossinae 58 F	*Brachyhesma (Brachyhesma) wyndhami*	1	2	3
				Colletidae	Hylaeus 6 F	*Hylaeus (Rhodohylaeus) proximus*	3		3
				Colletidae	Hylaeus 62 F	*Hylaeus (Rhodohylaeus) lateralis*	1	1	2
				Colletidae	Hylaeus 13 M	*Hylaeus (Prosopisteron) chlorosoma*		1	1
				Halictidae	Lasioglossum 36 F	*Lasioglossum (Chilalictus) cf. sexsetum*	1		1
			YellowBeeBowl_am	Diptera: Syrphidae	Syrphidae Sp.1	*Syrphidae Sp.1*	1		1
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	11		11
			YellowBeeBowl_pm	Colletidae	Euryglossinae 56 F	*Euhesma (Euhesma) balladonia/walkeri*	1		1
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	2		2
				Halictidae	Lasioglossum 33 F	*Lasioglossum (Chilalictus) cf. instabilis*	2		2
				Halictidae	Lasioglossum 34 F	*Lasioglossum (Chilalictus) cf. ptyon*	2		2
				Halictidae	Lasioglossum 36 F	*Lasioglossum (Chilalictus) cf. sexsetum*	2		2
	B (34)	[redacted]	BlueBeeBowl_am	Apidae	Amegilla 1 F	*Amegilla (Notomegilla) chlorocyanea*	1		1
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	5		5
				Megachilidae	Megachile 65 F	*Megachile 65 "prongs"*	1		1

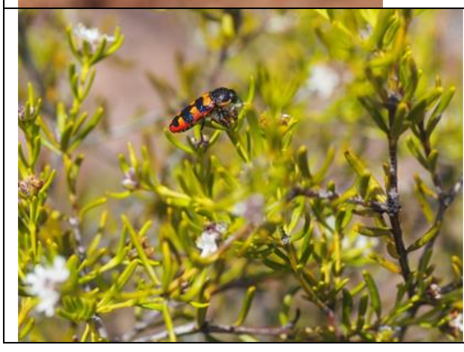
			YellowBeeBowl_am	Colletidae	Euryglossinae 55 F	*Euhesma (Euhesma) inconspicua*	1		1
				Colletidae	Euryglossinae 56 F	*Euhesma (Euhesma) balladonia/walkeri*	1		1
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	1		1
				Megachilidae	Megachile 65 F	*Megachile 65 "prongs"*	1		1
				Megachilidae	Megachile 69 F	*Megachile clypeata*	1		1
			YellowBeeBowl_pm	Coleoptera	Buprestidae sp.1	*Buprestidae sp.1*	1		1
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	4		4
				Megachilidae	Megachile 65 F	*Megachile 65 "prongs"*	2		2
	C (33)	[redacted]	BlueBeeBowl_am	Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	3	1	4
			BlueBeeBowl_pm	Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	5		5
				Halictidae	Lasioglossum 30 F	*Lasioglossum (Chilalictus) erythrurum spp-group*	3		3
				Halictidae	Lasioglossum 36 F	*Lasioglossum (Chilalictus) cf. sexsetum*	1		1
			Marianthus aquilonaris	**Megachilidae**	**Megachile 65 F**	***Megachile 65 "prongs"***	**1**		**1**
			YellowBeeBowl_am	Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	10		10
				Halictidae	Lasioglossum 32 F	*Lasioglossum (Chilalictus) cf. occiduum*	1		1
			YellowBeeBowl_pm	Halictidae	Lasioglossum 33 F	*Lasioglossum (Chilalictus) cf. instabilis*	1		1
				Megachilidae	Megachile 65 F	*Megachile 65 "prongs"*	1		1
	D (32, 41)	[redacted]	BlueBeeBowl_am	Diptera: Syrphidae	Syrphidae Sp.1	*Syrphidae Sp.1*	1		1
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	4		4
				Halictidae	Lasioglossum 26 F	*Lasioglossum (Chilalictus) cf. victoriellum*	1		1
				Halictidae	Lasioglossum 28 F	*Lasioglossum (Chilalictus) cf. greavesi*	1		1
				Halictidae	Lasioglossum 30 F	*Lasioglossum (Chilalictus) erythrurum spp-group*	1		1
				Halictidae	Lasioglossum 36 F	*Lasioglossum (Chilalictus) cf. sexsetum*	1		1
				Halictidae	Lipotriches 7 F	*Lipotriches (Austronomia) hippophila*	1		1
				Megachilidae	Megachile 65 F	*Megachile 65 "prongs"*	3		3

				Megachilidae	Megachile 67 F	*Megachile carnaua*	1		1
			BlueBeeBowl_pm	Coleoptera	Buprestidae sp.1	*Buprestidae sp.1*	1		1
				Halictidae	Lipotriches 2 F	*Lipotriches (Austronomia) flavovridis spp-group*	1		1
			Eucalyptus livida	Colletidae	Euryglossinae 61 F	*Xanthesma (Argohesma) nukarnensis*	1	11	12
				Colletidae	Hylaeus 13 F	*Hylaeus (Prosopisteron) chlorosoma*	1	1	2
				Colletidae	Euryglossinae 57 M	*Euhesma 57*		1	1
				Colletidae	Euryglossinae 62 F	*Indeterminate genus (undescribed, genus nov.) sp. nov.*	1		1
				Colletidae	Hylaeus 6 F	*Hylaeus (Rhodohylaeus) proximus*	1		1
				Colletidae	Hylaeus 62 F	*Hylaeus (Rhodohylaeus) lateralis*	1		1
				Halictidae	Lasioglossum 28 F	*Lasioglossum (Chilalictus) cf. greavesi*	1		1
				Halictidae	Lasioglossum 36 M	*Lasioglossum (Chilalictus) cf. sexsetum*	1		1
				Halictidae	Lasioglossum 36? F	*Lasioglossum (Chilalictus) cf. calophyllae*	1		1
				Halictidae	Lipotriches (Austronomia) australica F	*Lipotriches (Austronomia) australica*	1		1
			Marianthus aquilonaris	**Colletidae**	**Euryglossinae 60 M**	***Xanthesma sp***		**1**	1
			Marianthus aquilonaris	**Diptera: Syrphidae**	**Syrphidae Sp.1**	***Syrphidae Sp.1***	**1**		1
			Marianthus aquilonaris	**Halictidae**	**Lasioglossum 1 F**	***Lasioglossum (Chilalictus) castor***	**1**		1
			Marianthus aquilonaris	**Megachilidae**	**Megachile 66 F**	***Megachile 66 "shelf clypeus"***	**1**		1
			Marianthus aquilonaris	**Megachilidae**	**Megachile 70 M**	***Megachile maculosipes (WAM code 329)***		**1**	1
			Marianthus aquilonaris	**Diptera: Bombyliidae**	**Geron sp.1**	***Geron sp.1***	**2**		2
			YellowBeeBowl_am	Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	5		5
				Megachilidae	Megachile 65 F	*Megachile 65 "prongs"*	1		1
				Megachilidae	Megachile 66 F	*Megachile 66 "shelf clypeus"*	1		1
				Megachilidae	Megachile 68 M	*Megachile 68*		1	1

			YellowBeeBowl _pm	Diptera: Syrphidae	Syrphidae Sp.1	*Syrphidae Sp.1*	4		4
				Halictidae	Lasioglossum 1 F	*Lasioglossum (Chilalictus) castor*	9		9
				Megachilidae	Megachile 65 F	*Megachile 65 "prongs"*	5		5

Appendix E: Other plant species in flower

Table E1: Photos and species names of some of the plants in bloom

Image	ID
	*Scaevola spinescens*
	*Eremophila caperata*
	*Waitzia fitzgibbonii*
	*Asteridea athrixioides*
	*Westringia cephalantha*

	*Halgania lavandulacea*
	*Leptospermum incanum*
	*Alyogyne ?hakeifolia*

Appendix F: Native bee species photographs

Fig. F1: Male (above) and female (below) *Lasioglossum (Chilalictus) florale* visitors to *Marianthus aquilonaris*.

Fig. F2. Examples of native bee taxa sweepnetted from *Eucalyptus livida* (a-c) flowering in the vicinity of *Marianthus aquilonaris* a) *Xanthesma (Argohesma) nukarnensis*, female, b) *Brachyhesma (Brachyhesma) wyndhami*, female c) *Hylaues (Gnathopsis) amiculus*, female, and d) an undescribed *Megachile* collected in the bee bowls and from *Marianthus aquilonaris.*

Appendix G: Video file of native bees and other insects visiting *Eucalyptus livida* en masse

PB040044.MOV : https://drive.google.com/file/d/1sVFuR2lrh3WTbaSbZL95kfH-8gyf2Ys7/view?usp=sharing