A new species of mealybug (Hemiptera: Pseudococcidae) from critically endangered *Banksia montana* in Western Australia

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ABSTRACT – A new species of mealybug, *Pseudococcus markharveyi* Gullan sp. nov., is described and illustrated based on adult females collected from the critically endangered *Banksia montana* in the Stirling Range, Western Australia. A population of a possibly conspecific mealybug collected in 1985 from *B. heliantha* in Fitzgerald River National Park, Western Australia, has not been recollected despite intensive searches and may also be threatened. The new species is compared with similar mealybugs found in Australia and the Pacific region. Given the immediate threat to the host plant through multiple synergistic forces, namely plant disease, fire and climate change, the new mealybug species should be considered critically endangered.

KEYWORDS: Affiliate species, coendangered, coextinction, cothreatened, insect herbivores, plant-insect interactions, *Phytophthora cinnamomi*, short-range endemic species

INTRODUCTION

The Australian mealybug fauna (Hemiptera: Pseudococcidae) includes just over 200 named species in 66 genera (Ben-Dov 2012) and was documented comprehensively by Williams (1985) based almost entirely on collections available in museums. Subsequent collecting in natural areas has revealed many new mealybug species on native Australian plants (PJG, unpublished data).

As part of a project to determine the coextinction potential of plant-dwelling insects, mealybugs were collected on the foliage of *Banksia montana* (Proteaceae) in the Stirling Range National Park in southwest Western Australia. This *Banksia* species is one of the rarest in the genus with just 45 adult and 16 juvenile plants remaining in the wild by 2005 (Gilfillan *et al.* 2005). It is listed federally as Endangered and by the Western Australian State Government as Critically Endangered. Populations of *B. montana* are restricted to altitudes above 900 m in the eastern massif of the Stirling Range (Figure 1), and these populations have been decimated by the non-native plant pathogen *Phytophthora cinnamomi* (Barrett *et al.* 2008) and wildfires (Gilfillan *et al.* 2005). *Banksia montana* occurs within the federally-listed threatened ecological community called *Eastern Stirling Range Montane Heath and Thicket Community* (Figure 2).

Very similar mealybugs were collected in 1985 on *B. heliantha* at Fitzgerald River National Park. Both *Banksia* species formerly were included in *Dryandra* (Mast and Thiele 2007). The mealybugs have not been described previously and are most similar morphologically to several other Australian species presently placed in the large genus *Pseudococcus* Westwood, 1840. This paper describes and illustrates the new mealybug species from *B. montana* based on the adult females and discusses its biology, conservation status and relationships.

MATERIALS AND METHODS

Mealybugs were collected by hand from the foliage of the *Banksia* plants and preserved in 70% and 100% ethanol for transport to the laboratory. Preserved specimens were slide-mounted in Canada balsam using the method described in Gullan (1984), which is similar to the method of Williams and Granara de Willink (1992) except that xylene was used instead of clove oil. Adult females were mounted one specimen per slide. Most slide-mounts have been deposited in the Western Australian Museum (WAM), Perth, with a few paratype specimens in the Australian National Insect Collection (ANIC), CSIRO Ecosystem Sciences, Canberra.

The morphological terms used in the description are explained by Williams (1985) and Williams and Watson (1988). All measurements are maximum dimensions (e.g. body width and femur width were recorded at the widest

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FIGURE 1 Habitat of *Banksia montana* showing the eastern massif of the Stirling Range (photograph by M.L. Moir).

FIGURE 2 The Eastern Stirling Range Montane Heath and Thicket threatened ecological community at the summit of the eastern massif, featuring *B. montana* in the centre (photograph by M.L. Moir).

points) and are expressed as the range. Tarsal length excludes the claw. Spiracle length includes the muscle plate (apodeme). Setal lengths include the setal base. The illustration of the adult female represents a generalized individual based on several of the specimens used for the description. The central drawing shows the venter on the right and the dorsum on the left. Enlargements around the central drawing are not drawn to the same scale.

DNA was extracted from two adult females from the Stirling Range (see Material examined) and PCR and sequencing of the small subunit ribosomal RNA gene (18S) was conducted using the protocol described in Cook and Gullan (2004). Voucher specimens from the DNA work are housed in the ANIC.

After discovery of the mealybugs in the Stirling Range in 2007, and to determine its host specificity, a further 21 species of *Banksia*, six species of *Hakea*, five species of *Grevillea* and two species of *Petrophile* were sampled in the surrounding region. This additional sampling specifically included the only two sister taxa of *B. montana* (*B. pseudoplumosa, B. plumosa*, all are in the Series Plumosae: Cavanagh and Pieroni 2006), without discovering additional populations of the mealybug (Moir *et al*. 2012a). Furthermore, one of us (MCL) recently sampled >150 individuals of *B. heliantha*, in approximately the same locality at the same time of year as the collection in 1985, and did not locate any mealybug specimens.

SYSTEMATICS

Family Pseudococcidae Cockerell, 1905

Genus *Pseudococcus* Westwood, 1840

TYPE SPECIES

Dactylopius longispinus Targioni Tozzetti, 1867.

RELATIONSHIPS

According to the current taxonomy of Australian mealybugs (Williams 1985), the new species from *Banksia* would be placed in the large genus *Pseudococcus*. It has the following diagnostic features of *Pseudococcus*: 17 pairs of cerarii with each cerarius bearing two or three conical setae, trilocular pores and some with one or more auxiliary setae; enlarged dorsal and ventral ducts (probably oral-rim tubular ducts, see below); oral collar tubular ducts on the venter; and discoidal pores including adjacent to the rim of the enlarged ducts. However, the new species is unusual in lacking a circulus and multilocular pores, having typical oral-collar tubular ducts restricted to just a few near the vulva, having translucent pores restricted to the femur and tibia (none on the coxa) and possessing unusual enlarged tubular ducts dorsally and on the ventral margins. Each dorsal duct is 16.5–17.5 µm long and 8.0–12.5 µm wide, with the tube of the duct slightly thickened inwards from the rim for about one third of its length. It is not known whether these ducts are homologous with the oral-rim tubular ducts or oral-collar tubular ducts of typical mealybugs, and similar-looking dorsal ducts in other mealybug species have been referred to variously as drum-like tubular ducts, oral-collar or oral-rim ducts (Williams 1967, 1985; Beardsley 1971; Williams and Watson 1988; Williams 2004). Their structure is more consistent with oral-collar tubular ducts because they lack the distinct rim of oral-rim tubular ducts, however many of these unusual ducts have one or two minute discoidal pores on or near the rim of the duct orifice, which is characteristic of the oral-rim tubular ducts of some other *Pseudococcus* species. Here these ducts are referred to by the neutral descriptor 'drum-like', which is a term used by Beardsley (1971) and Williams (2004) in reference to similar enlarged ducts in *Tympanococcus* Williams, 1967.

Several endemic Australian *Pseudococcus* species share some features with the new Western Australian species. For example, the adult females of *P. anestios* Williams, 1985, *P. chenopodii* Williams, 1985, *P. epidendrus* Williams, 1985, *P. eremosus* Williams, 1985, and a group of five species found only on *Araucaria* (Araucariaceae) have dorsal oral-rim tubular ducts often with an obscure rim, translucent pores restricted to the hind femur and tibia, small oral-collar tubular ducts restricted to a few near the vulva, and multilocular pores totally absent (Williams 1985). Of these species, only *P. epidendrus* often has a discoidal pore associated with the rim of the dorsal ducts, and none of the these species have drum-like dorsal ducts.

Adult females of the genus *Tympanococcus* (with the type species from Hawaii and two others from the Philippines) and the endemic Hawaiian genus *Chlorococcus* Beardsley, 1971 (five species) share some features with the adult female of the new species described here, particularly the drum-like appearance of the dorsal ducts and the restriction of the translucent pores to the hind femur and tibia in most species (Zimmerman 1948; Beardsley 1959, 1963, 1971; Williams 2004). However all species of *Chlorococcus* and the type species of *Tympanococcus* possess a circulus, which is absent in the Western Australian species. However, like the Western Australian species, *Chlorococcus* species typically have one to three discoidal pores associated with the rim of the dorsal ducts and multilocular pores are either absent or just a few are present near the vulva. It is most likely that the similarity between *Chlorococcus* and the new Western Australian species is due to convergence. Several species of *Chlorococcus* are known only from above 1000 m in the Hawaiian mountains and the live females of all species are pale or bright green to yellowish-green and mostly live exposed on the leaves (Beardsley 1959, 1963, 1971). The new Western Australian species also occurs at a similar elevation (above 900 m), but live specimens are not green (see Description below). The three species of *Tympanococcus* have ventral multilocular pores, which are absent from the Western Australian species, fewer (3–14) pairs of cerarii than the Western Australian species, and the descriptions of *Tympanococcus* do not mention any discoidal pores associated with the dorsal ducts. However, like the Western Australian species, there are drum-like ventral ducts on adult females of *Tympanococcus* species, either confined to the body margin or more widely distributed. Living specimens of the type species of *Tympanococcus* are pale yellowish cream (Beardsley 1971). The genus *Mollicoccus* Williams, 1960, from the Solomon Islands (Williams and Watson 1988) has drum-like dorsal tubular ducts, similar to those of the new Western Australian species. However the dorsal tubular ducts of the only known species of *Mollicoccus*, *M. guadalcanalanus* Williams, 1960, are not associated with discoidal pores and the species also differs from the new species in lacking cerarii and in having multilocular disc pores and small oral-collar tubular ducts scattered on the abdomen and a few on the thorax.

Pseudococcus has more than 150 named species worldwide (Ben-Dov 2102) but molecular data suggest that the genus is not monophyletic (Hardy *et al.* 2008). However the relationships of sufficient numbers of species of *Pseudococcus* have not been studied for anyone to attempt to change the current functional classification of species in this and related genera. Some nucleotide sequence data from the nuclear small subunit ribosomal RNA gene (18S) were obtained for one specimen of the new species from each of the two sample sites in the Stirling Range and the two specimens were genetically identical. However there was insufficient molecular information on other Australian mealybugs to make any decision on relationships. Thus the conservative approach is followed here and the new species from *Banksia* is placed into *Pseudococcus* until further data are available on relationships.

Pseudococcus markharveyi **Gullan sp. nov.**

Figures 3, 4

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MATERIAL EXAMINED

Holotype

Australia: ***Western Australia*****:** adult female (1.85 mm long, 1.13 mm wide), Stirling Range National Park, Bluff Knoll, 34°22'51"S, 118°18'02"E, 27 November 2007, M. Moir, 'Pseudo15', on *Banksia montana* (WAM E83772).

Paratypes

Australia: ***Western Australia*****:** 2 adult females (2 slides), same data as holotype (1 in ANIC, 1 in WAM E83773); 3 adult females (including DNA voucher LGC01999: 3 slides), Stirling Range National Park, Bluff Knoll summit, 1039 m, 34°22'50.6"S, 118°15'02.1"E, 16 February 2012, M.C. Leng and F. Bokhari, 'Bluff Knoll B. mon. 8', on *B. montana* (1 in ANIC, 2 in WAM E83774 & E83775); 2 adult females (including DNA voucher LGC01998) and 1 embryo (3 slides), Stirling Range National Park, Pyungorup Peak, 1046 m, 34°21'33.8"S, 118°19'34.6"E, 14 February 2012, M.C. Leng and F. Bokhari, 'M004', on *B. montana* (1 in ANIC, 2 in WAM E3776 & E83777).

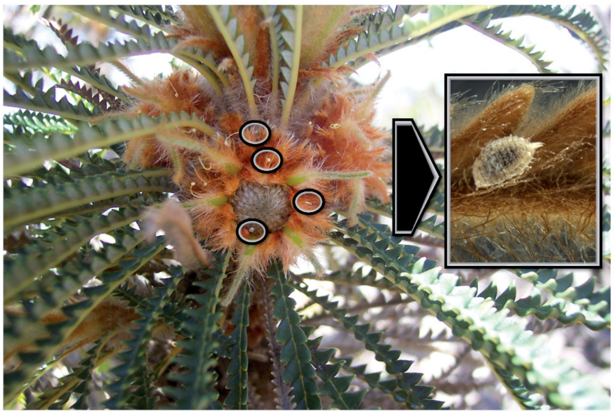

FIGURE 3 Mealybugs of *Pseudococcus markharveyi* sp. nov. (circled) in situ on the host plant *Banksia montana* (photographs by M.L. Moir).

Other material

Australia: ***Western Australia*****:** 2 adult females, Fitzgerald River National Park, c. 50 km SW of Ravensthorpe, 27 December 1985, C.A.M. Reid, on *Dryandra quercifolia* [now *Banksia heliantha*] (ANIC).

DIAGNOSIS

Type specimens of this new species have been collected only from the foliage of *B. montana* in southwest Western Australia. The slide-mounted adult female is characterised by having drum-like dorsal tubular ducts that often have 1 or 2 minute discoidal pores associated with the duct rim, slightly smaller and drum-like tubular ducts on ventral margin, 17 pairs of cerarii, each with 2 conical setae except first 2 pairs on head often with 3 conical setae, translucent pores confined to the femur and tibia, small ventral oral-collar tubular ducts confined to near the vulva, and by absence of a circulus and multilocular pores.

DESCRIPTION

Field features (Figure 3)

Mealybugs were found among the fine brown 'hairs' of *B. montana* on the main stem, undersides of leaves and developing flowers. Often they were crawling all over the leaves and developing flowers, but close to the main stem where the plant was the hairiest. Body colour pinkish with a covering of white wax.

Slide-mounted adult female (Figure 4)

(measurements based on 8 type specimens only)

Body 1.85–2.60 mm long, 1.13–1.65 mm wide;

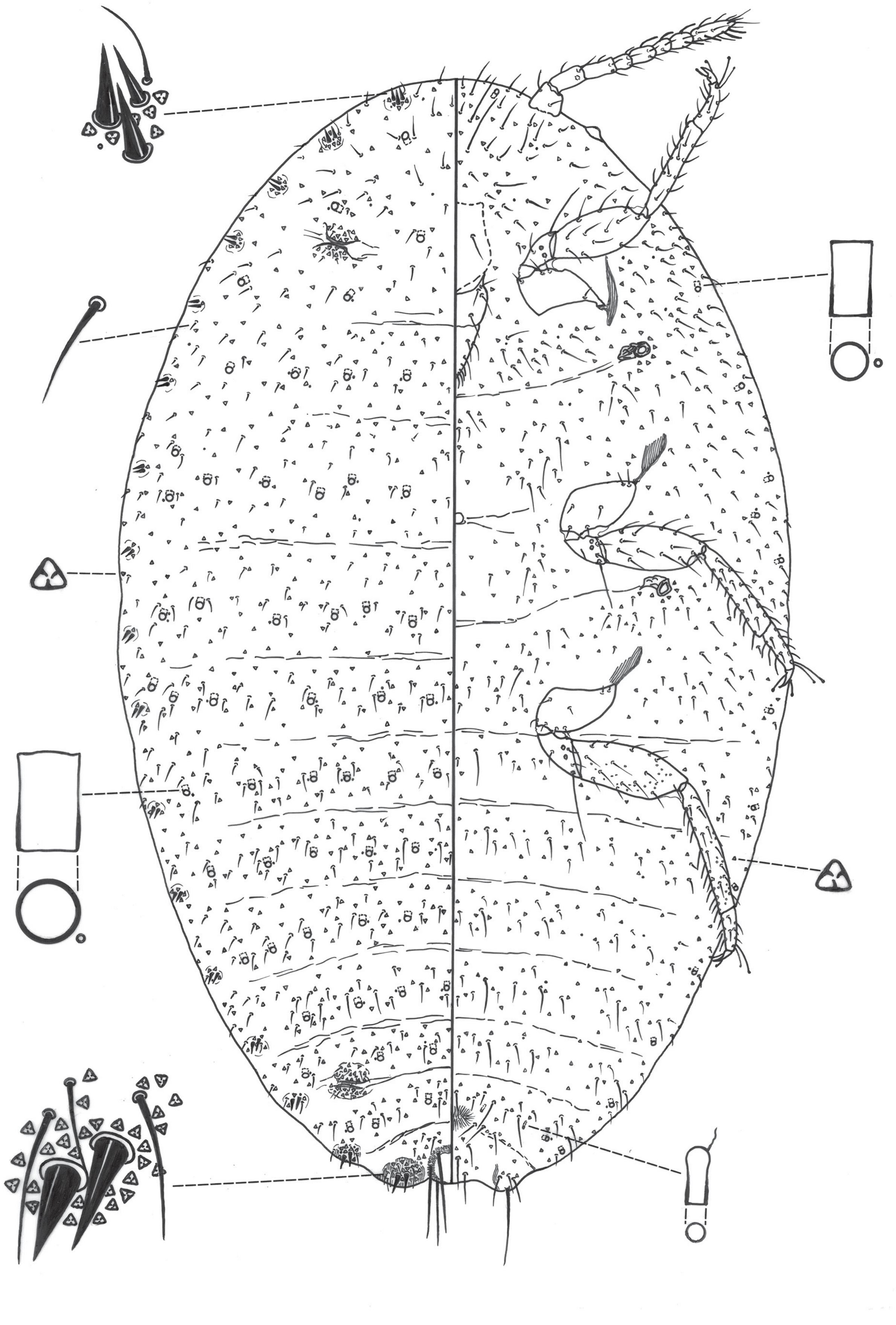

FIGURE 3 Adult female of *Pseudococcus markharveyi* sp. nov. (illustration by P.J. Gullan)

segmentation distinct. Eyespots 35–45 µm in diameter. Antennae (Figure 1a) 390–440 µm long, with 8 segments; apical segment 80–90 µm long, 26–35 µm wide. Labium 3 segmented, 170–190 µm long, 85–130 µm wide across base (often rotated). Clypeolabral shield (tentorium) 165–190 µm long. Mesothoracic spiracles 50–70 µm long including apodeme, 25–35 µm wide across peritreme; metathoracic spiracles 50–75 mm long including apodeme, 30–45 µm wide across peritreme. Legs well developed; hind legs with translucent pores dorsally on femur (17–32) and tibia (8–14), absent or rare on other segments; hind coxa 120–190 µm long, hind trochanter + femur 260–320 µm long, hind tibia + tarsus 335–380 µm long, hind femur 65–100 µm wide; hind trochanter with longest seta 110–130 µm long; tarsal digitules of each pair of legs capitate, but one shorter and thinner than other on all legs: 35–45 µm and 50–53 µm long; claw digitules capitate, 30–33 µm long; claw denticle not discernible. Ratio of lengths of hind tibia + tarsus to trochanter + femur 1.19–1.29; ratio of lengths of hind tibia to tarsus 2.4–3.1. Circulus absent. Both pairs of ostioles well developed; anterior ostioles 90–120 µm wide, each lip with 2–7 setae and 5–15 trilocular pores; posterior ostioles 100–120 µm wide, each lip with 4–8 setae and 11–21 trilocular pores. Anal ring 85–95 µm in outside width, with 6 anal ring setae 160–230 µm long. Anal lobes moderately developed, each with an elongate sclerotised area ventrally and an apical seta 125–150 µm long. Cerarii numbering 17 pairs, mostly each cerarius with 2 conical cerarian setae and a small cluster of trilocular pores (sometimes as few as one pore), but 1–3 auxiliary setae also in each cerarius on at least posterior abdomen and on head; each anal lobe cerarius with 2 subequal cerarian setae, 25–37.5 µm long, 9.0–12.5 µm wide at base, 4–6 auxiliary setae and 30–40 associated trilocular pores and 0–2 discoidal pores; penultimate cerarius with 2 usually subequal conical setae 23–33 µm long, more anterior cerarii with slightly shorter conical setae; first 2 pairs of cerarii on head (at base of antenna and near eye) each usually with 3 (rarely 2 or 4) conical cerarian setae and 0–2 auxiliary setae, 2–6 trilocular pores and 0–2 discoidal pores.

Dorsum: with flagellate setae, 10–38 µm long, sparsely distributed across all segments, longest on posterior abdominal segments and head. Drum-like tubular ducts 16.5–17.5 µm long and 8.0–12.5 mm wide, present in an irregular transverse row on each thoracic and abdominal segment and scattered on head; numbers as follows: 10–14 on head; on thoracic segments 6–12 on I, 9–18 on II and 11–15 on III; on abdominal segments 8–13 on I, 9–12 on II, 8–11 on III, 8–11 on IV, 7–9 on V, 3–6 on VI, 5–6 on VII and none on VIII. Trilocular pores 3.5–5.0 µm in diameter, scattered sparsely across all segments. Discoidal pores, ca. 2 µm in diameter, almost always associated with rim of drum-like tubular ducts, but also sparsely scattered on head. Multilocular pores and oral collar tubular ducts absent.

Venter: with flagellate setae 10–80 µm long, longest on head and posterior abdomen, sparsely distributed across all segments. Drum-like tubular ducts present marginally, ca. 15 µm long and 7.5–10.0 µm wide, smaller than on dorsum; distributed as follows on each side of body: 1–3 on head; 1–2 on each thoracic segment; 1–2 on each abdominal segment except none on segment VIII. Oral-collar tubular ducts 7.5–9.0 µm long, 3.5–4.0 mm wide at inner end, medially to submedially on abdominal segments V–VII; numbers as follows: 0–2 on V; 0–4 on VI; 1–4 on VII. Trilocular pores 3.2–5.0 µm in diameter, scattered across all segments, and usually with a linear group of up to 7 pores around atrium of each spiracle. Discoidal pores, size as on dorsum, scattered or near orifice of drum-like ducts. Multilocular pores absent.

Variation

One adult female from Pyungorup Peak has more poorly developed ostioles with fewer setae and pores, and slightly smaller spiracles and shorter legs than the other type specimens. The two adult females on *B. heliantha* from Fitzgerald River National Park differ from the adult females collected on the mountains in the Stirling Range in having a larger body (up to twice the size of the Stirling Range females), 25–66% longer antennae and legs, better developed ostioles (each lip of anterior ostioles with 5–8 setae and 14–22 pores; each lip of posterior ostioles with 7–12 setae and 22–30 pores), more pores (52–60) on each anal lobe cerarius, and usually two (rather than one) ventral oral-collar tubular ducts on each side of the anterior abdominal segments with one duct of each pair smaller than the other. Due to this variation, these two females have been excluded from the type series and their meristic and mensural features are excluded from the description above. It is possible that the morphological differences observed between specimens from the Stirling Range National Park and the more coastal Fitzgerald River National Park may result from different developmental temperatures experienced by the mealybugs. The edges of the two parks are less than 100 km apart but separated largely by agricultural land. Further collecting in this intervening region as well as in the two parks may help to establish if the two populations are: (1) isolated geographically, (2) restricted in host-plant preference, and (3) morphologically and genetically distinct.

ETYMOLOGY

The specific name is in honour of Dr Mark Harvey, who has been a pioneer in the discovery and conservation for short-range endemic invertebrates in the biodiversity hotspot of southwest Western Australia.

DISCUSSION

Pseudococcus markharveyi was discovered in 2007 through targeted sampling of threatened plant species and non-threatened congenerics (and named *Pseudococcus* sp. 15 in Moir *et al.* 2012a,b). The fate of the mealybug was brought into question (Moir *et al.* 2012a) because of the tenuous nature of the remaining natural populations of *B. montana* (Gilfillan *et al.* 2005), and the ex-situ conservation methods currently employed to assist in saving the host species (seed storage and translocations: Coates and Atkins 2001; Gilfillan *et al.* 2005).

Whether *P. markharveyi* is monophagous on the critically endangered *B. montana* is questionable given a possible collection on *B. heliantha*. Further collecting is planned in the near future to try to secure specimens from *B. heliantha* for molecular comparison with the Stirling Range specimens of *P. markharveyi.*

Taking the precautionary principle that *B. montana* is the only host, *P. markharveyi* should be considered critically endangered. This conclusion is supported by applying the decision framework for cothreatened taxa of Moir *et al.* (2011). It should be noted that populations of *B. montana* may survive, but still result in the extinction of the mealybug. This early extinction of the mealybug species could occur when the host population becomes too small to sustain a viable population of mealybugs (see Moir *et al.* 2010). The level at which an organism will go extinct due to a change in some required variable (e.g., number of habitat patches) has been termed the extinction threshold in studies of metapopulations (e.g., Benton 2003). Furthermore, the future survival of *P. markharveyi* is not secured should subsequent populations be discovered on other hosts. Fitzpatrick *et al.* (2008) recently predicted that many species of *Banksia* will be extinguished or undergo significant range retractions given climate change. *Banksia montana* was not considered by these authors, but it is restricted to the highest altitudes of the highest mountain range in southwest Western Australia. Due to its isolated distribution, *B. montana* has nowhere to migrate to if climate warms and would no doubt become extinct (taxa at high altitudes face higher risk of extinction: Thomas *et al.* 2011). In contrast, *B. heliantha* was considered by Fitzpatrick *et al.* (2008) and is predicted to undergo range expansion under most climate-change scenarios. However, we note that the predicted expansion is dependent upon no intervention of synergistic forces. For example, the plant pathogen *Phytophthora cinnamomi* is in Fitzgerald River National Park, although currently the majority of the park is disease-free (Dunne et al. 2011), and most *Banksia* species are very susceptible to this pathogen (Shearer *et al*. 2007).

Of the five species of Hemiptera (true bugs) listed by the IUCN Red List as extinct, two are mealybugs: *Clavicoccus erinaceus* Ferris in Zimmerman (1948) and *Phyllococcus oahuensis* (Ehrhorn) (World Conservation Monitoring Centre 1996a, 1996b; International Union for Conservation of Nature 2012). Both species probably became extinct on Hawaii due to the reduction in their host plant populations from habitat loss (*C. erinaceus* feeds on the threatened plant *Abutilon sandwicense* and *P. oahuensis* on two species of *Urera*; Beardsley 1984), which is similar to the threatening process affecting *P. markharveyi*.

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