



Diversity and host specificity of Psylloidea (Hemiptera) inhabiting box mistletoe, *Amyema miquelii* (Loranthaceae) and three of its host *Eucalyptus* species

Anna E Burns,^{1*} Gary S Taylor,² David M Watson¹ and Saul A Cunningham³

¹*School of Environmental Sciences and Institute for Land, Water and Society, Charles Sturt University, PO Box 789, Albury, NSW 2640, Australia.*

²*Australian Centre for Evolutionary Biology and Biodiversity, School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia.*

³*CSIRO Ecosystem Sciences, Black Mountain, ACT 2601, Australia.*

Abstract

This study is the first direct comparison of the diversity of phytophagous insects associated with a parasitic plant and its host plants. Specifically, we compared the species composition, density and host specificity of psylloids or jumping plant lice (Hemiptera: Psylloidea), inhabiting hemiparasitic box mistletoe *Amyema miquelii*, and three of its host *Eucalyptus* species: *Eucalyptus blakelyi*, *Eucalyptus melliodora* and *Eucalyptus polyanthemos*. Insects were sampled by restricted canopy fogging in remnant *Eucalyptus* woodlands in an agricultural region of temperate south-eastern Australia. Although most psylloids are understood to be mono- or oligophagous, most species in our survey were found on the foliage of both mistletoes and eucalypts. Nevertheless, analysis of density patterns and reference to previous work on psylloids supports the high degree of host specificity for psylloids, leading to distinct assemblages on these two intimately associated plants. We show that (1) there were two mistletoe-associated species of psylloid and 18 eucalypt-associated species; (2) there were a large number of tourist species, as indicated by known psylloid/plant host associations; and (3) psylloid density was higher on eucalypt than mistletoe leaves. The different psylloid assemblages found on box mistletoes compared with their host plants are likely to be due to differences in the foliar properties implicated in host specificity and host selection by phytophagous insects. Further research is required to understand the ecological dynamics and evolutionary origins of these arboreal assemblages.

Key words arboreal, assemblage similarity, community composition, Psyllidae, tourist species.

INTRODUCTION

Studies of host specialisation of insects inform our understanding of species richness and turnover among host plants and locations and, thus, species diversity at different scales (Ødegaard *et al.* 2000; Dyer *et al.* 2007; Novotny *et al.* 2007). Variation in community composition among insect assemblages is strongly influenced by host plant identity or plant species composition, particularly for phytophagous insects (Frenzel & Brandl 2001; Ødegaard *et al.* 2005). Mistletoes are parasitic plants that are physiologically linked with their host plants, and thereby strongly spatially associated, such that mistletoes and host plants share the same physical environment and may appear to be one plant canopy (especially in cases of leaf mimicry by mistletoes, see Mathiasen *et al.* 2008). If plant chemistry and evolutionary history were not important, one would expect the assemblage of insects on

mistletoe to be a nested subset of the assemblage on the host plant. However, mistletoes and their host plants usually belong to different orders and clades. The association between mistletoe and host plant offers an opportunity to explore factors that influence the host specificity, composition and distribution of arboreal arthropod assemblages in tree canopies.

Although interactions between mistletoes and vertebrates have been well studied (as reviewed by Watson 2001; Watson & Herring 2012), much less is known about invertebrate assemblages associated with mistletoes. Species-specific studies have shown that ecological interactions between mistletoes and insects include herbivory, frugivory, pollination and tri-trophic relationships (Penfield *et al.* 1976; Atsatt 1981; De Baar 1985; Braby 2000, 2005; French 2004; Robertson *et al.* 2005). However, we are unaware of any direct comparative studies of arthropod assemblages on mistletoes and their host plants, apart from our own research (Burns 2009; Burns *et al.* 2011), and one indirect investigation of a food plant shift of a lepidopteran between a dwarf mistletoe and host conifers (Mooney 2003).

*aeburns78@gmail.com

The mistletoe form has evolved five times in different families within the Santalales (Vidal-Russell & Nickrent 2008) and mistletoe species vary in their degree of host specificity (Barlow 1996; Downey 1998). Australian mistletoes, of which there are 91 species, belong to the families Loranthaceae (in 12 genera, Gondwanan origin), Viscaceae (in 3 genera, Laurasian origin) and Santalaceae (1 genus/species) and constitute approximately 6% of nearly 1600 species worldwide (Barlow 1984; Downey 1998; Downey & Wilson 2004; Nickrent 2011; Watson 2011). Our study species, the box mistletoe, *Amyema miquelii* (Loranthaceae), is distributed throughout mainland Australia and has been recorded parasitising 125 host plant species, primarily in *Eucalyptus* (Myrtaceae; Downey 1998; Watson 2011). It was the most common mistletoe species in the study area. The three *Eucalyptus* species included in this study (see Materials and Methods) are widely distributed in south-eastern Australia, in woodlands and open forest (Chippendale 1988).

Psylloidea (Hemiptera) were the most abundant group of insects collected in a previous study of the arthropod assemblages inhabiting mistletoes and eucalypts (Burns *et al.* 2011). Here, we examine the diversity and distribution of psylloids from that survey. Psylloidea are particularly diverse and abundant in Australia, where the 382 described species (and >100 species not yet described) constitute more than 10% of the world fauna (Hollis 2004; and recent taxonomic descriptions, including Taylor *et al.* 2010, 2013; Taylor & Kent 2013; Taylor & Moir 2014). Currently, three described species of psylloids have been collected from Australian mistletoes (listed as ‘*Amyema pendulum*’, ‘probably *A. miquelii*’ and ‘unidentified species’; see Taylor 1999) and many species from *Eucalyptus* (Hollis 2004). However, few community-level studies have described the assemblages of psylloids on particular host plant species (Hodkinson *et al.* 1979; Jäger & Topp 2002; Moir *et al.* 2005, 2011; Powell 2011; Powell *et al.* 2012), including only one for a *Eucalyptus* species (Barton *et al.* 2013) and none for mistletoes. Therefore, our aim was to investigate the diversity and host specificity of psylloids inhabiting box mistletoe and a subset of its host plants. Given that most psylloid species are either monophagous or oligophagous, i.e. feeding and completing their life cycle on a single host plant species or a group of congeneric or confamilial species (Hodkinson 1974; Hollis 2004), we predicted low similarity in species composition between box mistletoe and the eucalypts, despite the close physical association of these plants.

MATERIALS AND METHODS

Study area and sampling protocol

The study was conducted in the south-west slopes region of New South Wales, Australia (near Holbrook: 35°44’S, 147°19’E), where woodland and forest have been extensively cleared for agriculture and remaining habitat is restricted to a series of disjunct remnants. Sampling occurred in

Table 1 Details of the sampling design, including the number of samples (*n*) collected from individual box mistletoe (*Amyema miquelii*) plants and *Eucalyptus* trees, and the number of solo and duplicate mistletoe samples (i.e. solo = no sample collected from the host tree; duplicate = two mistletoe samples collected in the same tree)

Site No.	Mistletoe <i>n</i>	Eucalypt <i>n</i>	<i>Eucalyptus</i> species (<i>n</i>)	Solo mistletoe samples	Duplicate mistletoe samples
1	4	3	RB(3)	0	1
2	6	5	RB(2), YB(2), BR(1)	1	0
3	5	3	RB(2), YB(1)	0	2
4	10	6	RB(3), YB(1), BR(2)	3	1
5	5	2	RB(2)	2	1
Totals	30	19	RB(12), YB(4), BR(3)	6	5

RB: red box, *E. polyanthemus*; YB: yellow box, *E. melliodora*; BR: Blakely’s red gum, *E. blakelyi*.

November 2005 at five sites of remnant grassy box woodland, 4–18 hectares in size and located 2–23 km apart (i.e. sites 1–5 in Burns *et al.* 2011). These sites were surrounded by grazed pastures or crops on privately owned land. The remnant woodlands were dominated by *Eucalyptus polyanthemus* (red box), which is the primary host of *A. miquelii* in the region. At least 40 individual *A. miquelii* occurred at each site.

Insects were collected using a restricted canopy fogging technique (after Basset 1990), from plants of *A. miquelii* (*n* = 30) and three of its host tree species: *E. polyanthemus* (red box, *n* = 12), *Eucalyptus melliodora* (yellow box, *n* = 4) and *Eucalyptus blakelyi* (Blakely’s red gum, *n* = 3) using a trailer-mounted hydraulic bucket hoist (maximum height: 12 m above ground). Sampling was constrained by the tree species mix of each remnant patch and the access permitted by the hoist; therefore, it was not possible to collect the same number of samples from each *Eucalyptus* species. The greater number of samples collected from box mistletoe than the eucalypts occurred for two reasons: in some cases, mistletoe foliage was accessible but the tree foliage was out of reach (six trees); or, two mistletoe plants were sampled in the same tree (five trees, see Table 1). At each site, sampled trees were between 5 and 500 m apart. Psylloids were sampled from eucalypt foliage within approximately 3 m of a mistletoe plant that was sampled in the same tree, at a similar height above ground and depth in the canopy (3 m was within the reach of the boom arm of the bucket hoist without moving its base). Psylloids were collected from one sample of foliage per mistletoe plant or eucalypt tree. The dimensions of the sampled foliage were recorded (i.e. height, width and depth) and a density score was assigned. The volume of the sample was calculated using the equation for an ellipsoid and multiplied by the density score. A subsample of foliage of box mistletoe and each eucalypt species was removed, after measuring the dimensions, and leaf area was measured. The relationship between foliage volume and leaf area was calculated for each plant species and the regression equations were used to estimate the leaf area of all samples

(see Burns *et al.* 2011 for further details). Hence, the abundance of psylloids is expressed as density of individuals per estimated leaf area (m²).

Adult psylloid specimens were sorted to morphospecies by A.E.B. and identified to species by G.S.T. The family : sub-family classification follows Burckhardt and Ouvrard (2012). Voucher specimens of Psylloidea have been deposited in the Australian National Insect Collection (Canberra). The probable host specificity of the psylloid taxa was assessed from host records of conspecific or congeneric taxa in the literature (primarily from Hollis 2004) and indicator species analysis (described below). Indicator analysis is useful for inferring the fidelity of taxa to predefined groups based on the frequency and abundance of taxa (e.g. Progar & Schowalter 2002). Taxa that were identified by these means as host specific or strongly associated with the host genera were deemed to be transient or tourist species (*sensu* Moran & Southwood 1982) when they were collected on non-host foliage. However, these nominations should be regarded as provisional and should be assessed by life history studies of the psylloid taxa.

Data analyses

Given the small number of samples obtained from each of the three *Eucalyptus* species, we pooled the data and treated assemblages found on the three different *Eucalyptus* species as one 'Eucalyptus associated' assemblage for comparison with the psylloid assemblages from *A. miquelii* plants (subsequently referred to as eucalypt and mistletoe samples, respectively). This approach allowed detection of host association at the genus level, but ignores the possibility of associations with specific *Eucalyptus* species.

Indicator species analysis (Legendre & Legendre 1998; McCune & Grace 2002) was used to detect host plant preferences of the psylloid taxa by determining whether each taxon had a non-random distribution with regard to host plant genus, i.e. *Amyema* or *Eucalyptus*. The indicator species values (*IV*) are based on the average relative abundance (as density of individuals per leaf area, in this study) and frequency of the taxa in each group of samples: $IV_{kj} = (\text{Relative abundance}_{kj} \times \text{Relative frequency}_{kj}) \times 100$ for taxa *j* in host group *k* (McCune & Grace 2002). To test the null hypothesis that the highest indicator value (*IV*_{max}) for taxa *j* in host group *k* is no larger than would be expected by chance, a Monte Carlo randomisation test was used, with 4999 permutations of the data matrix, in PC-ORD 5.07 © (McCune & Mefford 2006). The *P* value refers to the proportion of randomised trials with an indicator value equal to or exceeding the observed indicator value.

The species richness of the psylloid assemblages associated with the mistletoes and eucalypts was estimated using the non-parametric Chao 1 estimator (Chao 1984; Colwell & Coddington 1994), which uses abundance data and incorporates the observed number of species plus a ratio of rare species in the samples (i.e. singletons and doubletons). We used species densities rounded to the nearest whole number (required for this analysis with EstimateS 7.5.1 ©, Colwell

2006); hence, singletons and doubletons were species represented by a density of one or two individuals per square metre, respectively. Tourist species were excluded from analyses, as were samples without any psylloid specimens; therefore, the total number of samples was 16 eucalypt (*E. polyanthemos*: *n* = 11; *E. melliodora*: *n* = 3; *E. blakelyi*: *n* = 2), and 29 mistletoe. To compare similarity in species composition and abundance among and between mistletoe and eucalypt samples, we calculated Bray–Curtis similarities on untransformed species densities and estimated 'true' similarity using the Jaccard abundance-based estimator (Chao *et al.* 2005), which takes into account the effect of unseen shared species. The uneven sampling of mistletoes and eucalypts was dealt with by excluding mistletoe samples without a paired eucalypt sample and, in the case of duplicate mistletoes within a tree, by selecting the mistletoe sample located closest to the sampled eucalypt foliage. This resulted in 19 paired samples. Species richness and similarity indices were calculated with the default settings in EstimateS (Colwell 2006). The observed and estimated similarity between the psylloid assemblages showed similar patterns; therefore, only the latter are shown in the results (Fig. 3).

To test whether dissimilarity in species composition and abundance of psylloid assemblages was greater between than among the mistletoe and eucalypt sample groups, we used the non-parametric statistical test: multi-response permutation procedure (MRPP, Legendre & Legendre 1998) with the Bray–Curtis dissimilarity index in PC-ORD 5.07 © (McCune & Mefford 2006). The only assumption of MRPP – spatial independence of sample units – was assessed using a Mantel Test (PC-ORD 5.07). The spatial distances between samples were calculated from the geographical coordinates of the samples (obtained with a handheld GPS). The correlation between spatial distance and community dissimilarity of the samples did not differ significantly from zero (Mistletoe samples: Mantel *r* = -0.09, *P* = 0.3; Eucalypt samples: Mantel *r* = -0.08, *P* = 0.5); therefore, the samples were considered spatially independent.

RESULTS

Twenty-one psylloid morphospecies belonging to 12 genera were collected and eight of these were identified to species (see Table 2 and further details about each species provided in Table S1: the term 'species' will be used to refer to both described species and undetermined morphospecies). Three species belong to *Acizzia* (Psyllidae: Acizzinae), 17 species to 10 genera in the Aphalaridae: Spondylaspidinae and one species to *Schedotrioza* (Trioziidae) (Table 2). Sixteen psylloid species occurred on both box mistletoe and eucalypt foliage, four were found only on eucalypts and one was found only on box mistletoe (Table 2). The average density of psylloids was greater on eucalypt foliage than mistletoe foliage (Table 2). The greatest density of any one species (i.e. *Australopsylla* sp. 1) was 33 individuals per m² of foliage on *E. melliodora* and 16 individuals per m² across all eucalypts

Table 2 Species inventory of psylloid assemblages collected from box mistletoe (*Amyema miquelii*) and three host *Eucalyptus* species. This includes a comparison of the average density (number of individuals per m² leaf area, dry weight) and percent frequency of psylloid species collected from box mistletoe, each *Eucalyptus* species and the three *Eucalyptus* species combined (*N.B.* The density of individuals was calculated from the area of both sides of the leaves). The species richness totals include tourist/transient species (see text for further details)

Host plant	<i>Amyema miquelii</i> (n = 30)		All <i>Eucalyptus</i> samples (n = 19)		<i>Eucalyptus polyanthemos</i> (n = 12)		<i>Eucalyptus melliodora</i> (n = 4)		<i>Eucalyptus blakelyi</i> (n = 3)	
	Density	Frequency	Density	Frequency	Density	Frequency	Density	Frequency	Density	Frequency
<i>Acizzia loranthacae</i> (Psyllidae)	2.99	87	0.04	5	0.1	8	0	0	0	0
<i>Acizzia amyemae</i> (Psyllidae)	0.98	63	0.03	5	0.1	8	0	0	0	0
<i>Acizzia</i> sp. 1 (Psyllidae)	0	0	0.04	5	0	0	0.2	25	0	0
<i>Anoeconeossa communis</i> (Aphalaridae)	0.04	10	0.49	53	0.6	58	0.4	50	0.3	33
<i>Anoeconeossa</i> sp. 1 (Aphalaridae)	0.01	3	0.16	26	0.2	25	0.2	50	0	0
<i>Australopsylla</i> sp. 1 (Aphalaridae)	2.67	57	15.77	63	13.9	83	33.3	17	0	0
<i>Australopsylla</i> sp. 2 (Aphalaridae)	1.20	37	10.17	47	6.9	58	27.6	50	0	0
<i>Australopsylla</i> sp. 3 (Aphalaridae)	0	0	0.01	5	0.1	8	0	0	0	0
<i>Blastopsylla</i> sp. 1 (Aphalaridae)	0.01	3	0.25	32	0.3	42	0	0	0.2	33
<i>Blastopsylla</i> sp. 2 (near <i>multisetulae</i> Taylor, 1985) (Aphalaridae)	0.02	7	0.45	16	0.5	8	0.7	50	0	0
<i>Cardiaspina albicollaris</i> (Aphalaridae)	0.01	3	0.06	11	0.1	17	0	0	0	0
<i>Cardiaspina retator</i> (Aphalaridae)	0.01	3	0.02	5	0	0	0.1	25	0	0
<i>Creiis</i> sp. (Aphalaridae)	0	0	0.07	5	0	25	0.3	25	0	0
<i>Ctenarytaina</i> sp. (Aphalaridae)	0.03	7	0.05	11	0.1	17	0	0	0	0
<i>Glycaspis</i> sp. (Aphalaridae)	0.08	17	0.22	16	0.2	17	0.4	50	0.3	33
<i>Hyalinaspis</i> sp. (Aphalaridae)	0.01	3	0	0	0	0	0	0	0	0
<i>Phyllolyma</i> sp. (Aphalaridae)	0.01	3	0.02	5	0.1	8	0	0	0	0
<i>Platyobria adustalata</i> (Aphalaridae)	0.10	17	0.16	21	0.2	25	0.1	25	0	0
<i>Platyobria lewisi</i> (Aphalaridae)	0.04	10	0.13	16	0.1	17	0.3	25	0	0
<i>Platyobria</i> sp. 1 (Aphalaridae)	0	0	0.06	11	0.1	8	0	0	0.2	33
<i>Schedotrioza distorta</i> (Triozidae)	0.01	3	0.01	5	0.1	8	0	0	0	0
Average density (all psylloid species)	9.2		28.22		23.7		63.6		1.0	
Species richness	17		20		17		11		4	

combined (Table 2). At least half of the species in the study occurred at a density of less than 0.1 individual per m² of foliage (Fig. 1) and were represented by less than 10 individuals. Considering both mistletoe and eucalypt assemblages, two species were represented by only two individuals (i.e. doubletons) and seven species by only one individual (i.e. singletons).

Two of the most abundant and frequently occurring psylloid species on box mistletoe, *Acizzia loranthacae* Taylor and *Acizzia amyemae* Taylor (Table 2, Fig. 2), are considered host specific to *Amyema* mistletoes (Taylor 1999). These data are

the first confirmed host records of these two species on *A. miquelii*. Furthermore, these two species were significantly more abundant and frequent on box mistletoe than the eucalypts and, thus, were identified as indicator species of box mistletoe (Table 3). Only one *A. amyemae* and two *A. loranthacae* occurred in the eucalypt samples; therefore, they are considered tourist species on the eucalypts. The single individual of an undescribed species of *Acizzia* (*Acizzia* sp. 1, Table 2) that occurred on *E. melliodora* was also deemed a tourist because *Eucalyptus* is not recorded as a host for this genus (Hollis 2004; Taylor & Moir 2014). *Amyema*, *Acacia*,

Fig. 1. The relative abundance of psyllid species inhabiting box mistletoe and the eucalypts, comparing assemblages with all species to those with host-specific species only (i.e. those considered to be host-specific to either *Amyema* or *Eucalyptus*). □, Eucalypts – all psyllid species; ■, Eucalypts – host-specific species; ▤, Box mistletoe – all psyllid species; ▥, Box mistletoe – host-specific species.

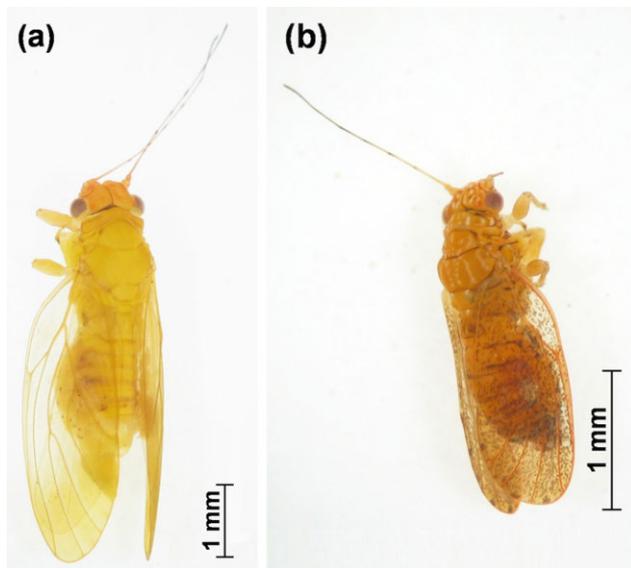
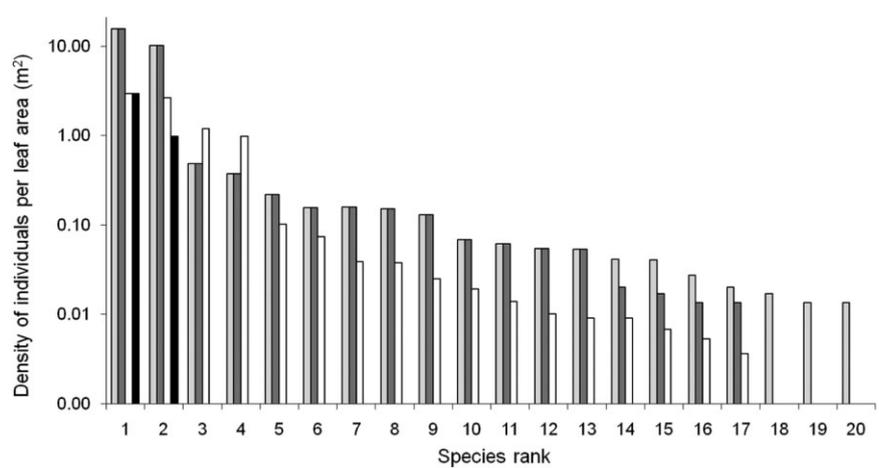


Fig. 2. Psyllid taxa (Hemiptera: Psyllidae) collected from box mistletoe (*Amyema miquelii*): (a) *Acizzia loranthacae*, (b) *Acizzia amyemae*.

Grevillea and *Hakea* are known host genera for *Acizzia* species (Hollis 2004; Taylor & Moir 2014); therefore, to be conservative, *Acizzia* sp. 1 was treated as unassigned to a host genus in this study.

The other psyllid taxa (identified to species or genus) belong to genera that have been recorded on *Eucalyptus* species or other Myrtaceous taxa (Table S1). Five of these (*Anoeconeossa communis*, *Australopsylla* sp. 1 and 2, *Anoeconeossa* sp. 1, *Blastopsylla* sp. 1) occurred at significantly greater frequency and density on eucalypts than mistletoes and, thus, were identified as indicator species of the eucalypts in this study (Table 3). Given that psyllids are usually mono- or oligophagous, and that all of the remaining taxa are recorded from *Eucalyptus* (indeed that the entire subfamily Spondyliaspidae feeds almost exclusively on Myrtaceae, Hollis 2004), they can be provisionally nominated as eucalypt associated, and considered tourists on mistletoes.

Table 3 Psyllid species with significant indicator species values ($P < 0.05$), based on the abundance (density of individuals per m^2 leaf area) and frequency of the species in the mistletoe and eucalypt samples ($n = 30$ and $n = 19$, respectively)

Species	Indicated host†	Indicator value (%)‡	P-value
<i>Acizzia loranthacae</i>	Mistletoe	85	0.0002
<i>Acizzia amyemae</i>	Mistletoe	61	0.0012
<i>Anoeconeossa communis</i>	Eucalypts	55	0.0002
<i>Anoeconeossa</i> sp. 1	Eucalypts	28	0.0044
<i>Australopsylla</i> sp. 1	Eucalypts	61	0.0340
<i>Australopsylla</i> sp. 2	Eucalypts	48	0.0366
<i>Blastopsylla</i> sp. 1	Eucalypts	34	0.0022

†The group of samples (mistletoes vs. eucalypts) that contains the maximum indicator value for each psyllid species.

‡Derived from multiplication of the average relative abundance and average relative frequency of each psyllid species among the mistletoe samples and the eucalypt samples. Values shown are for the identified indicator group.

Based on this provisional assessment of host plant associations, the observed species richness of the assemblages were two species on box mistletoe, 18 on the eucalypts, and one (*Acizzia* sp. 1) on neither host taxa. The estimated species richness of the psyllid assemblage inhabiting box mistletoe was also two species because *Acizzia amyemae* and *A. loranthacae* (the two mistletoe-associated species) occurred at densities of greater than two individuals per square metre (*N.B.* the 95% CI of the Chao 1 mean was zero after five samples and, therefore, is not shown on Fig. 3). The estimated species richness of the psyllid assemblage on the eucalypts was 21 (95% CI: 18–43, Chao1 index, Fig. 3).

The similarity between the psyllid assemblages on box mistletoe and the eucalypts was reduced from 30% when tourist species were included (for paired samples in the same tree, and 17% for all possible pairwise sample combinations), to zero similarity when tourist species were excluded from the assemblages (Fig. 4). Thus, assemblage dissimilarity between the mistletoe and eucalypt sample groups was statistically significant ($P < 0.0001$, MRPP). Assemblage similarity was greater among mistletoe assemblages than among the eucalypt

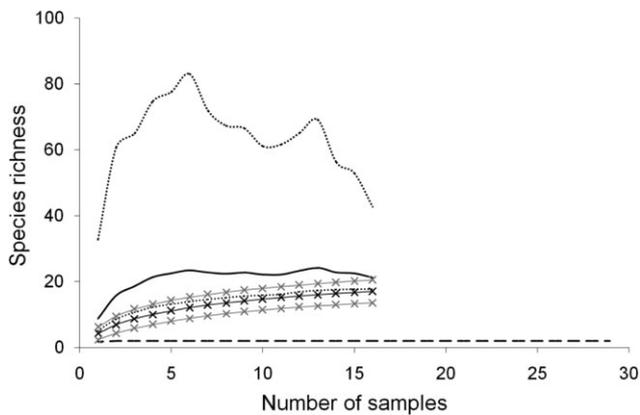


Fig. 3. Species accumulation curves for observed and estimated species richness of the psyllid assemblages inhabiting *Eucalyptus* host plants (estimated richness, Chao 1 mean: solid black line and 95% confidence interval: dotted lines; observed richness: crossed black line and 95% CI: crossed grey lines; $n = 16$) and *Amyema miquelii* (estimated richness, Chao 1 mean: dashed black line; $n = 29$). *N.B.* For the psyllid assemblage on mistletoes, the observed species richness is not shown because it was very similar to the estimated richness, and the 95% CI of the Chao 1 mean is not shown because it was zero after five samples. Tourist species were excluded.

assemblages (Fig. 4). This would be due to the occurrence of fewer species on the mistletoes and less variation in density of individuals compared with the eucalypt assemblages (Table 2).

DISCUSSION

Composition and similarity of the psyllid assemblages

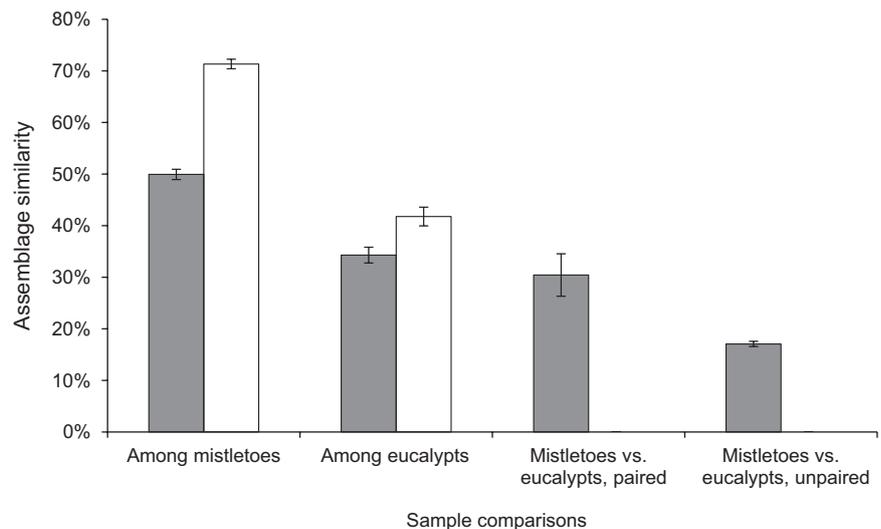
We found that the box mistletoes and eucalypt trees hosted different species assemblages of psyllids, after determination of probable host specificity. Furthermore, the species richness and overall density of psyllids was greater on eucalypts than mistletoes. This was not only a consequence of sampling three species of eucalypts vs. one species of mistletoe, because at least 10 species of psyllids occurred on each of two of the eucalypt species. The differences in diversity may be related to the abundance and apparency of host plants in the landscape. Thus, plant identity was a key determinant of the community composition of the psyllid assemblages.

Our results suggest complete dissimilarity between the ‘host-specific’ psyllid assemblages inhabiting box mistletoe and the host eucalypts, based on the assumption that psyllids are primarily mono- or oligophagous and are genetically constrained in host selection and utilisation (van Klinken 2000; Hollis 2004). The species richness of the psyllid assemblage on box mistletoe was quite low, with only two species of *Acizzia*. We found a third *Acizzia* species, but it was at low density and found only on eucalypt foliage. One other species of *Acizzia* (*Acizzia pendulae*) occurs on *Amyema pendula*, which is closely related to *A. miquelii* (Barlow 1984). Addi-

tional sampling and life history studies are required to determine whether any of these *Acizzia* species inhabit and can complete their life cycle on other *Amyema* species. The host *Eucalyptus* species of box mistletoe were not exhaustively sampled, as reflected by the wide confidence interval of the Chao 1 richness estimator. To accurately determine the host specificity of the psyllid taxa on *Eucalyptus*, life history studies are required with a variety of host plants. This would clarify whether or not host plant shifts have occurred for any of the psyllid species found on box mistletoe and its host eucalypts. The psyllid species inhabiting *Amyema* mistletoes are more closely related to those inhabiting *Acacia* than *Eucalyptus* species (Taylor 1999; Yen 2002). This may indicate an ancient radiation of psyllids from *Acacia* to mistletoes on *Acacia* and thence to mistletoes in the canopy of *Eucalyptus* trees (Taylor 1999). Host switching by psyllids is thought to be constrained by host plant foliage, thus resulting in phylogenetically conservative host plant radiations (Percy *et al.* 2004). Recent examples have shown host switching among closely related (i.e. congeneric) hosts. A specific example includes the apparent recent switch of *Acizzia solanicola* Kent & Taylor (2010) from native *Solanum*, rock nightshade (*Solanum petrophilum*), to an introduced weed, wild tobacco tree (*Solanum mauritianum*), and commercial solanaceous crops such as eggplant (*Solanum melongena*) (Taylor & Kent 2013). Furthermore, studies of the foliage chemistry of mistletoes and their host plants could help to elucidate the causal factors relating to the patterns of psyllid radiations and host switching.

The dissimilarity between the ‘host-specific’ psyllid assemblages was initially masked by the large proportion of tourist species on the mistletoes (i.e. 90% of species). The high frequency of tourist psyllids on the mistletoes is most likely to be due to a ‘mass effect’, i.e. ‘the establishment of species in sites where they cannot be self-maintaining’ (Shmida & Wilson 1985, p. 1), which is influenced by the reproductive success and dispersal ability of species in adjacent areas, the heterogeneity of the biota and environmental conditions in the sampled area (Shmida & Wilson 1985). The greater mass of eucalypt foliage and density of eucalypt-inhabiting psyllids in our study area would have facilitated the greater occurrence of tourist species on the mistletoe plants than the eucalypts. Box mistletoes constitute 2–20% of canopy biomass of eucalypts, on average, in woodlands of this study area (Cooney & Watson 2005; March & Watson 2007), which was consistent with our study sites. Furthermore, our study sites had relatively low plant species diversity (A Burns unpublished data 2005). Thus, it appears that the close proximity of mistletoe and eucalypt foliage, the higher density of eucalypt-inhabiting psyllid populations compared with mistletoe-inhabiting psyllid populations and the much larger canopy volume of eucalypts relative to mistletoes led to more tourist psyllids occurring on the mistletoes. This phenomenon would be facilitated by both active and passive dispersal of adult psyllids, which can jump a few metres in the air and disperse longer distances by wind (Hodkinson 1974; Hollis 2004). The observation of greater similarity between assemblages of psyllids

Fig. 4. Mean (\pm 1SE) percentage similarity in species composition and abundance (Jaccard abundance-based estimator) of the psyllid assemblages with and without tourist species (grey and white bars, respectively), among and between the mistletoe and eucalypt samples. Paired samples from mistletoe and eucalypt foliage were collected within the same tree. (Number of pairwise comparisons for each sample group: mistletoes 400, eucalypts 136, mistletoe vs. eucalypts paired 19, mistletoe vs. eucalypts unpaired 510).



collected in the same tree, compared with that of all tree–mistletoe combinations, further supports the occurrence of a mass effect. In this sense, the assemblage of psyllids on the parasitic mistletoe includes a nested subset of the host eucalypt tree, but the species in this subset are unlikely to be feeding on the mistletoe foliage.

The importance of determining tourist or transient species on host plants has been recognised since the beginning of canopy diversity studies because it influences estimates of species richness and guild composition and, thus, comparisons of diversity and functional groups among plant species, ecosystems, countries and continents (Moran & Southwood 1982; Stork 1987; Ødegaard 2006). In some diversity studies, singleton species are excluded because of the risk that they represent tourist species; however, host-specific, rare species are also excluded by this practice. Indeed, the number of singletons and doubletons can be used to estimate species richness (e.g. Chao 1). Therefore, we suggest that a preferable approach is to determine actual or probable host specificity of target taxa before analysing the diversity and similarity of assemblages. Tourist species have been found to constitute 10–70% of insect species in tree canopies (Moran & Southwood 1982; Stork 1987; Basset & Arthington 1992; Majer *et al.* 2000; Jäger & Topp 2002; Ødegaard 2004; Southwood *et al.* 2005). However, not all canopy studies determine the host specificity of the herbivorous insects collected, especially for adult Coleoptera or Lepidoptera (e.g. Cunningham *et al.* 2005). This is partly dependent on the form of insect collection. Direct collection from plant foliage and canopy fogging provide more useful information about the host specificity of study taxa than do light or Malaise trapping, for example. Inference about host specificity is also improved when ecological surveys sample across different plant species, allowing for probabilistic methods of host identification, such as indicator species analysis. Feeding trials, larval rearing and/or statistical techniques are useful when host plant records and life history information are inadequate to determine host specificity (e.g. Novotny *et al.* 2002; Vesik *et al.* 2010). The next steps for our research

would be to obtain species-level identifications for the morphospecies we collected from *Eucalyptus* and more life history information to verify host records and, thus, increase the accuracy of host specificity and the diversity indices.

Differences in density of psyllids between host plants

The pattern of greater density of psyllids on eucalypts than mistletoes could be due to underlying differences in foliar properties. The concentration of total nitrogen in the foliage of the eucalypt species in this study was significantly greater than that of box mistletoe (Burns *et al.* 2011). It is well known that nitrogen is a limiting nutrient for the growth and development, and hence, abundance of animals (White 1993). Several studies have found that the abundance of insects, including sap-sucking bugs such as psyllids, is positively correlated with foliar nutrient concentrations (e.g. Basset 1991; Recher *et al.* 1996; Tassone & Majer 1997; Peeters 2002). Furthermore, in another study of psyllid populations inhabiting eucalypts, peaks in the availability of foliar nitrogen coincided with peaks in the abundance of psyllid adults and particular developmental stages of nymphs (Taylor 1997). The study by Taylor (1997) also showed that some psyllid species are adapted to low nitrogen availability in host plant foliage. Therefore, it is highly likely that differences in foliar nutrients of the plants in this study influenced the observed variation in density and composition among the psyllid assemblages. This is an area requiring further research at a finer scale, e.g. identification and quantification of particular amino acids that influence the density of psyllids on their host plants (Steinbauer 2013).

CONCLUSION

This study indicates that psyllid assemblages inhabiting box mistletoes are of low diversity but high host specificity. The close spatial and biological association between box

mistletoes and their host eucalypt trees contributed to a high incidence of tourist or transient species in the psylloid assemblages inhabiting the mistletoe plants but there was no evidence for species sharing the two host genera. Further research is required to verify the host specificity of the psylloid taxa and understand species radiations among host plants. It is likely that differences in foliar properties between the mistletoes and eucalypt, including low nitrogen availability in mistletoe, influenced variation in the density of the psylloids and might restrict host switching.

ACKNOWLEDGEMENTS

This research was conducted while A.E.B was the recipient of an Australian Post-graduate Award. Additional funding was received from the Holsworth Wildlife Research Endowment and the Ecological Society of Australia (student grant). Preparation of the manuscript was assisted by a Writing-up Award from Charles Sturt University to A.E.B. The authors would like to thank the landowners for access to their properties, and Matthew Herring and the Ecology Lab of CSU for field assistance. The manuscript was improved by suggestions from Dr Rieks van Klinken.

REFERENCES

- Atsatt PR. 1981. Ant-dependent food plant selection by the mistletoe butterfly *Ogyris amaryllis* (Lycaenidae). *Oecologia* **48**, 60–63.
- Barlow BA. 1984. Loranthaceae. In: *Flora of Australia Volume 22 Rhizophorales to Celastrales* (ed. AS George), pp. 68–130. Australian Government Publishing Service, Canberra.
- Barlow BA. 1996. Advances in systematic knowledge of Australian Loranthaceae and Viscaceae: a review. *Telopea* **6**, 851–862.
- Barton PS, Colloff MJ, Pullen KR & Cunningham SA. 2013. Arthropod assemblages in a focal tree species (*Eucalyptus microcarpa*) depends on the species mix in restoration plantings. *Biodiversity and Conservation* **22**, 2091–2110.
- Basset Y. 1990. The arboreal fauna of the rainforest tree *Argyrodendron actinophyllum* as sampled with restricted canopy fogging: composition of the fauna. *The Entomologist* **109**, 173–183.
- Basset Y. 1991. Influence of leaf traits on the spatial distribution of insect herbivores associated with an overstorey rainforest tree. *Oecologia* **87**, 388–393.
- Basset Y & Arthington AH. 1992. The arthropod community of an Australian rainforest tree: abundance of component taxa, species richness and guild structure. *Australian Journal of Ecology* **17**, 89–98.
- Braby MF. 2000. *Butterflies of Australia: Their Identification, Biology and Distribution*. CSIRO Publishing, Melbourne.
- Braby MF. 2005. Afrotropical mistletoe butterflies: larval food plant relationships of *Myllothris* Hubner (Lepidoptera: Pieridae). *Journal of Natural History* **39**, 499–513.
- Burckhardt D & Ouvrard D. 2012. A revised classification of the jumping plant-lice (Hemiptera: Psylloidea). *Zootaxa* **3509**, 1–34.
- Burns AE. 2009. *Diversity and dynamics of the arthropod assemblages inhabiting mistletoe in eucalypt woodlands*. PhD thesis, Charles Sturt University.
- Burns AE, Cunningham SA & Watson DM. 2011. Arthropod assemblages in tree canopies: a comparison of orders on box mistletoe (*Amyema miquelii*) and its host eucalypts. *Australian Journal of Entomology* **50**, 221–230.
- Chao A. 1984. Non-parametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* **11**, 265–270.
- Chao A, Chazdon RL, Colwell RK & Shen TJ. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecology Letters* **8**, 148–159.
- Chippendale GM. 1988. *Eucalyptus, Angophora* (Myrtaceae). In: *Flora of Australia Volume 19* (ed. AS George), pp. 1–540. Australian Government Publishing Service, Canberra.
- Colwell RK. 2006. *EstimateS*: Statistical estimation of species richness and shared species from samples. User's guide and application. [Accessed 16 April 2008.] Available from URL: <http://purl.oclc.org/estimates>
- Colwell RK & Coddington JA. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions: Biological Sciences* **345**, 101–118.
- Cooney SJN & Watson DM. 2005. Diamond firetails (*Stagonopleura guttata*) preferentially nest in mistletoe. *Emu* **105**, 317–322.
- Cunningham SA, Floyd RB & Weir TA. 2005. Do *Eucalyptus* plantations host an insect community similar to remnant *Eucalyptus* forest? *Austral Ecology* **30**, 103–117.
- De Baar M. 1985. The complex mistletoe-insect community. *The Entomological Society of Queensland Bulletin* **13**, 100–102.
- Downey PO. 1998. An inventory of host species for each aerial mistletoe species (Loranthaceae and Viscaceae) in Australia. *Cunninghamia* **5**, 685–720.
- Downey PO & Wilson CA. 2004. *Muellerina flexilabastra* (Loranthaceae): a new species of mistletoe from south-eastern Australia. *Australian Systematic Botany* **17**, 441–445.
- Dyer LA, Singer MS, Lill JT *et al.* 2007. Host specificity of Lepidoptera in tropical and temperate forests. *Nature* **448**, 696–U699.
- French JA. 2004. Ecological interactions between western hemlock dwarf mistletoe (*Arceuthobium tsugense* subsp. *tsugense*) and insects within an old-growth forest. Master of Science, University of Washington.
- Frenzel M & Brandl R. 2001. Hosts as habitats: faunal similarity of phytophagous insects between host plants. *Ecological Entomology* **26**, 594–601.
- Hodkinson ID. 1974. The biology of the Psylloidea (Homoptera): a review. *Bulletin of Entomological Research* **64**, 325–339.
- Hodkinson ID, Jensen TS & Maclean SF. 1979. Distribution, abundance and host plant relationships of *Salix*-feeding psyllids (Homoptera, Psylloidea) in arctic Alaska. *Ecological Entomology* **4**, 119–132.
- Hollis D. 2004. *Australian Psylloidea: Jumping Plantlice and Lerp Insects*. Australian Biological Resources Study, Canberra.
- Jäger S & Topp W. 2002. Jumping plant-lice species associated with willow trees on the floodplains of the Rhine river (Homoptera: Psylloidea). *Entomologia Generalis* **26**, 47–64.
- Legendre P & Legendre L. 1998. *Numerical Ecology*, 2nd English edn. Elsevier, Amsterdam, the Netherlands.
- Majer JD, Recher HF & Ganesh S. 2000. Diversity patterns of eucalypt canopy arthropods in eastern and western Australia. *Ecological Entomology* **25**, 295–306.
- March WA & Watson DM. 2007. Parasites boost productivity: effects of mistletoe on litterfall dynamics in a temperate Australian forest. *Oecologia* **154**, 339–347.
- Mathiasen RL, Nickrent DL, Shaw DC & Watson DM. 2008. Mistletoes: pathology, systematics, ecology, and management. *Plant Disease* **92**, 988–1006.
- McCune B & Grace J. 2002. *Analysis of Ecological Communities*. MjM Software Design, Gleneden Beach, Oregon, USA.
- McCune B & Mefford MJ. 2006. PC-ORD. Multivariate Analysis of Ecological Data. MjM Software, Gleneden Beach, Oregon, U.S.A.
- Moir ML, Brennan KEC, Fletcher MJ, Majer JD & Koch JM. 2011. Multi-scale patterns in the host specificity of plant-dwelling arthropods: the influence of host plant and temporal variation on species richness and assemblage composition of true bugs (Hemiptera). *Journal of Natural History* **45**, 2577–2604.
- Moir ML, Brennan KEC, Majer JD, Fletcher MJ & Koch JM. 2005. Toward an optimal sampling protocol for Hemiptera on understorey plants. *Journal of Insect Conservation* **9**, 3–20.
- Mooney KA. 2003. *Promylea lumigerella glendella* Dyar (Pyralidae) feeds on both conifers and parasitic dwarf mistletoe (*Arceuthobium* spp.): one example of food plant shifting between parasitic plants and their hosts. *Journal of the Lepidopterists' Society* **57**, 47–53.

- Moran VC & Southwood TRE. 1982. The guild composition of arthropod communities in trees. *The Journal of Animal Ecology* **51**, 289–306.
- Nickrent DL. 2011. Santalales (including mistletoes). In: *Encyclopedia of Life Sciences (ELS)*. John Wiley & Sons, Ltd, Chichester. DOI: 10.1002/9780470015902.a0003714.pub2 [Accessed 17 September 2014.] Available from: <http://www.els.net/WileyCDA/ElsArticle/refId-a0003714.html>
- Novotny V, Basset Y, Miller SE, Drozd P & Cizek L. 2002. Host specialization of leaf-chewing insects in a New Guinea rainforest. *Journal of Animal Ecology* **71**, 400–412.
- Novotny V, Miller SE, Hulcr J *et al.* 2007. Low beta diversity of herbivorous insects in tropical forests. *Nature* **448**, 692–U698.
- Ødegaard F. 2004. Species richness of phytophagous beetles in the tropical tree *Brosimum utile* (Moraceae): the effects of sampling strategy and the problem of tourists. *Ecological Entomology* **29**, 76–88.
- Ødegaard F. 2006. Host specificity, alpha- and beta-diversity of phytophagous beetles in two tropical forests in Panama. *Biodiversity and Conservation* **15**, 83–105.
- Ødegaard F, Diserud OH, Engen S & Aagaard K. 2000. The magnitude of local host specificity for phytophagous insects and its implications for estimates of global species richness. *Conservation Biology* **14**, 1182–1186.
- Ødegaard F, Diserud OH & Ostbye K. 2005. The importance of plant relatedness for host utilization among phytophagous insects. *Ecology Letters* **8**, 612–617.
- Peeters PJ. 2002. Correlations between leaf constituent levels and the densities of herbivorous insect guilds in an Australian forest. *Austral Ecology* **27**, 658–671.
- Penfield FB, Stevens RE & Hawksworth FG. 1976. Pollination ecology of three Rocky Mountain dwarf mistletoes. *Forest Science* **22**, 473–484.
- Percy DM, Page RDM & Cronk QCB. 2004. Plant-insect interactions: double-dating associated insect and plant lineages reveals asynchronous radiations. *Systematic Biology* **53**, 120–127.
- Powell FA. 2011. Can early loss of affiliates explain the coextinction paradox? An example from *Acacia*-inhabiting psyllids (Hemiptera: Psylloidea). *Biodiversity and Conservation* **20**, 1533–1544.
- Powell FA, Hochuli DF, Symonds CL & Cassis G. 2012. Are psyllids affiliated with the threatened plants *Acacia ausfeldii*, *A. dangarensis* and *A. gordonii* at risk of co-extinction? *Austral Ecology* **37**, 140–148.
- Progar RA & Schowalter TD. 2002. Canopy arthropod assemblages along a precipitation and latitudinal gradient among Douglas-fir *Pseudotsuga menziesii* forests in the Pacific Northwest of the United States. *Ecography* **25**, 129–138.
- Recher HF, Majer JD & Ganesh S. 1996. Eucalypts, arthropods and birds: on the relation between foliar nutrients and species richness. *Forest Ecology and Management* **85**, 177–195.
- Robertson AW, Ladley JJ & Kelly D. 2005. Effectiveness of short-tongued bees as pollinators of apparently ornithophilous New Zealand mistletoes. *Austral Ecology* **30**, 298–309.
- Shmida A & Wilson MV. 1985. Biological determinants of species diversity. *Journal of Biogeography* **12**, 1–20.
- Southwood TRE, Wint GRW, Kennedy CEJ & Greenwood SR. 2005. The composition of the arthropod fauna of the canopies of some species of oak (*Quercus*). *European Journal of Entomology* **102**, 65–72.
- Steinbauer MJ. 2013. Shoot feeding as a nutrient acquisition strategy in free-living psyllids. *PLoS ONE* **8**, e77990.
- Stork NE. 1987. Guild structure of arthropods from Bornean rain-forest trees. *Ecological Entomology* **12**, 69–80.
- Tassone RA & Majer JD. 1997. Abundance of arthropods in tree canopies of *Banksia* woodland on the Swan Coastal Plain. *Journal of the Royal Society of Western Australia* **80**, 281–286.
- Taylor GS. 1997. Effect of plant compounds on the population dynamics of the lerp insect, *Cardiaspina albitextura* Taylor (Psylloidea: Spondyliaspidae) on Eucalypts. In: *Ecology and Evolution of Plant-Feeding Insects in Natural and Man-made Environments* (ed. A Raman), pp. 37–57. International Scientific Publications, New Delhi.
- Taylor GS. 1999. New species of *Acizzia* Heslop-Harrison (Hemiptera: Psyllidae) from Australian mistletoe (Loranthaceae). *Australian Journal of Entomology* **38**, 66–71.
- Taylor GS, Austin AD, Jennings JT, Purcell MF & Wheeler GS. 2010. *Casuarinicola*, a new genus of jumping plant lice (Hemiptera: Triozidae) from *Casuarina* (Casuarinaceae). *Zootaxa* **2601**, 1–27.
- Taylor GS, Jennings JT, Purcell MF & Austin AD. 2013. Three new Australian species of *Trioza* Foerster (Hemiptera: Triozidae) with a remarkable adaptation to oviposition on articulate branchlets of *Allocasuarina* (Casuarinaceae). *Australian Journal of Entomology* **52**, 42–52.
- Taylor GS & Kent DS. 2013. Potential economic pests of solanaceous crops: a new species of *Solanum*-feeding psyllid from Australia and first record from New Zealand of *Acizzia solanicola* (Hemiptera: Psyllidae). *Zootaxa* **3613**, 257–273.
- Taylor GS & Moir ML. 2014. Further evidence of the co-extinction threat for jumping plant-bugs: three new co-threatened *Acizzia* (Psyllidae) and *Trioza* (Triozidae) from Western Australia. *Insect Systematics and Evolution* **45**, 283–302.
- van Klinken RD. 2000. Host-specificity constrains evolutionary host change in the psyllid *Prosopidopsylla flava*. *Ecological Entomology* **25**, 413–422.
- Vesk PA, McCarthy MA & Moir ML. 2010. How many hosts? Modelling host breadth from field samples. *Methods in Ecology and Evolution* **1**, 292–299.
- Vidal-Russell R & Nickrent DL. 2008. The first mistletoes: origins of aerial parasitism in Santalales. *Molecular Phylogenetics and Evolution* **47**, 523–537.
- Watson DM. 2001. Mistletoe – a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics* **32**, 219–249.
- Watson DM. 2011. *Mistletoes of Southern Australia*. CSIRO Publishing, Collingwood.
- Watson DM & Herring M. 2012. Mistletoe as a keystone resource: an experimental test. *Proceedings of the Royal Society B-Biological Sciences* **279**, 3853–3860.
- White TCR. 1993. *The Inadequate Environment: Nitrogen and the Abundance of Animals*. Springer-Verlag, Berlin.
- Yen AL. 2002. Short-range endemism and Australian Psylloidea (Insecta: Hemiptera) in the genera *Glycaspis* and *Acizzia* (Psyllidae). *Invertebrate Systematics* **16**, 631–636.

Accepted for publication 24 August 2014.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1 Information about the Psylloidea taxa collected from box mistletoe (*Amyema miquelii*) and three of its *Eucalyptus* hosts (*E. polyanthemus*, *E. melliodora* and *E. blakelyi*) sourced from Taylor (1999) and Hollis (2004) (identification by G.S.T.).