International Journal for Parasitology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Novel application of species richness estimators to predict the host range of parasites

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ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form 30 September 2016
Accepted 5 October 2016
Available online xxxx

Keywords:
Host range
Host specificity
Sampling
Results-based stopping rule
Parasitic plant
Mistletoe

ABSTRACT

Host range is a critical life history trait of parasites, influencing prevalence, virulence and ultimately determining their distributional extent. Current approaches to measure host range are sensitive to sampling effort, the number of known hosts increasing with more records. Here, we develop a novel application of results-based stopping rules to determine how many hosts should be sampled to yield stable estimates of the number of primary hosts within regions, then use species richness estimation to predict host ranges of parasites across their distributional ranges. We selected three mistletoe species (hemiparasitic plants in the Loranthaceae) to evaluate our approach: a strict host specialist (*Amyema lucasii*, dependent on a single host species), an intermediate species (*Amyema quandang*, dependent on hosts in one genus) and a generalist (*Lysiana exocarpi*, dependent on many genera across multiple families), comparing results from geographically-stratified surveys against known host lists derived from herbarium specimens. The results-based stopping rule (stop sampling bioregion once observed host richness exceeds 80% of the host richness predicted using the Abundance-based Coverage Estimator) worked well for most bioregions studied, being satisfied after three to six sampling plots (each representing 25 host trees) but was unreliable in those bioregions with high host richness or high proportions of rare hosts. Although generating stable predictions of host range with minimal variation among six estimators trialled, distribution-wide estimates fell well short of the number of hosts known from herbarium records. This mismatch, coupled with the discovery of nine previously unrecorded mistletoe-host combinations, further demonstrates the limited ecological relevance of simple host-parasite lists. By collecting estimates of host range of constrained completeness, our approach maximises sampling efficiency while generating comparable estimates of the number of primary hosts, with broad applicability to many host-parasite systems.

1. Introduction

When sampling any group of organisms, it has long been noted that sampling effort confounds estimates of species richness (Magurran, 2004; Chao and Jost, 2012). A comparable issue arises in parasitology, where sampling effort influences estimates of both host range (the number of host species infected by a particular parasite) and parasite species richness (number of parasite species that infect a particular host; Poulin, 1992; Walther et al., 1995; GuÕgan and Kennedy, 1996; Walther and Morand, 1998). Estimates of host range increase with the number of individual hosts examined per host species, and with the number of surveys per parasite species (Walther et al., 1995). Estimated host range is also confounded by distributional extent: as more of the geographic distribution of the parasite is sampled, the greater the proportion of host species that are encountered (e.g., metazoan parasites in freshwater fish; Poulin, 1992).

To minimise the confounding effects of sampling effort and generate reliable and comparable estimates of host range, sampling effort needs to be standardised (Poulin, 1992; Walther et al., 1995; Grenfell and Burns, 2009; Krasnov et al., 2011). Standardisation methods vary in their approach and suitability to a given context. Extrapolation methods compare completeness of sampled sets (Walther et al., 1995; Watson, 2003; Chao and Jost, 2012). Post hoc methods (rarefaction) can be applied to data already collected (Grenfell and Burns, 2009; Kavanagh and Burns, 2012) to remove the confounding factor of sampling effort. This approach to standardisation necessarily results in removal of data to ensure equivalent completeness across sample targets (Watson, 2003) and

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http://dx.doi.org/10.1016/j.ijpara.2016.10.001
0020-7519/

therefore the loss of potentially biologically meaningful information. Loss of data is more problematic for small datasets (including rare species that are infrequently encountered). Also, this approach may not be appropriate where areas rather than individuals have been sampled or where individuals are clumped (Smith and van Belle, 1984), as is often the case with parasite data. Even where sampling effort bias may be corrected using post hoc extrapolation, the collection bias (especially for herbarium and other museum specimens) remains (Downey, 1998; Norton and De Lange, 1999; Grenfell and Burns, 2009; Kavanagh and Burns, 2012).

Watson (2003, 2010) developed the standardised search to estimate species richness of birds, combining whole-of-patch sampling with results-based stopping rules to generate richness estimates of constrained and comparable completeness. Results-based stopping rules shift emphasis away from the effort expended and focus on accuracy and comparability of results (Peterson and Slade, 1998; Hopps, 2012). Some a priori knowledge of the system under study is required as sample methods and stopping rule are chosen before sampling commences. A robust rule defining the precision of sampling completeness required to estimate richness is determined, then applied during sampling and when the data satisfy the rule, sampling is complete (Watson, 2003). Hence, the effort applied to sample each study site is immaterialby calculating richness estimates iteratively, effort is scaled to sample completeness, sampling all sites to the same pre-determined degree of precision. In addition to ensuring comparability of estimates, this approach maximises sampling efficiency.

Although species richness estimators have previously been applied to host-parasite data (Walther and Morand, 1998), three aspects of our work are novel. Firstly, we believe this is the first application of an iterative results-based stopping rule in real time to maximise the reliability and efficiency of estimating the number of species hosting a particular parasite. Second, unlike otherwise similar applications of results-based stopping rules for birds (Watson, 2010; Luck et al., 2013), bryophytes (Callaghan, 2012) and licence plates (Peterson and Slade, 1998) to determine whether additional samples are required to estimate species richness at that locality, the stopping rule is here applied spatially to decide whether additional localities within a bioregion need to be sampled to derive robust richness estimates. Finally, treating bioregional host inventories of known completeness as samples, estimates of host range across the distributional range of parasites were derived and compared with previously published inventories of known hosts to determine reliability. Although using mistletoe species as illustrative examples, the techniques developed here are broadly relevant to studies of host-parasite dynamics generally, representing a tractable approach to estimate host range free from sampling bias.

2. Materials and methods

2.1. Study species

Mistletoes are an ideal system for studying the interplay between sampling effort and host range. Most parasites are more difficult to sample, often requiring capture and careful examination of hosts, magnifying the impact of improvements to sampling efficiency and reliability of host range estimates on study design and logistics. As aerial hemiparasites, both host and parasite are sessile and the parasite is readily discernible (Reid, 1990; Overton, 1994; Mathiasen et al., 2008), allowing accurate estimates of the abundance and density of mistletoes, hosts and potential hosts. The taxonomy of Australian mistletoes is well-resolved and field identification of species is straightforward (Watson, 2011).

From Downeys (1998) inventory of all known hosts for Australian mistletoe species drawn from herbarium specimens, three mistletoe species were selected representing narrow (dependent on a single host species), intermediate (dependent on host species in one genus) and broad (dependent on many host genera across multiple families) host ranges. Leopardwood mistletoe *Amyema lucasii* (Blakely) Danser is considered to be almost exclusively dependent on leopardwood (*Flindersia maculosa*) hosts (Barlow, 1984; Cunningham et al., 1992; Quirico, 1992; Watson, 2011) but has been recorded on a further six host species (Barlow, 1984; Downey, 1998; Watson, 2011), five of which occur in the family Rutaceae. It is found in semi-arid woodland from the Mitchell District, Queensland, Australia to the lower Darling River, New South Wales (NSW), Australia (Barlow, 1984; Fig. 1A). Grey mistletoe *Amyema quandang* (Lindl.) Tiegh. has been recorded on 53 host species, of which 40 are in the *Acacia* genus with *Acacia dealbata, Acacia pendula, Acacia harpophylla, Acacia homalophylla, Acacia aneura* and *Acacia papyrocarpa* commonly recorded as hosts (Barlow, 1984; Reid and Lange, 1988; Quirico, 1992; Keith, 2004; Barea and Herrera, 2009; Bowen et al., 2009; Watson, 2011). *Amyema quandang* occurs in semi-arid and arid woodland in all mainland states of Australia (Barlow, 1984; Fig. 1B). Harlequin mistletoe *Lysiana exocarpi* (Behr) Tiegh. has been recorded infecting 114 species in 45 genera and 21 families (Downey, 1998; Watson, 2011), with the most common hosts belonging to the genera *Acacia, Senna, Casuarina, Eremophila, Alectryon, Exocarpos, Santalum* and *Amyema* (epiparasitic on the latter three parasitic genera in the Santalales). It has the largest range of the study species (including eight exotic species) and grows in open forest and woodland in arid to temperate regions of all mainland states in Australia (Barlow, 1984; Fig. 1C). For these three mistletoe species, available host lists (Downey, 1998) representing a comprehensive inventory of all plant species known to host these mistletoes were used to compare predictions based on geographically-stratified surveys (Milner, K. 2014. Optimising estimates of host spectrum: Australian mistletoe as a model system. Honours thesis (unpublished), University of Technology, Sydney, Australia).

2.2. Mistletoe surveys

Field sampling was conducted in AprilSeptember 2014 across six of the 17 bioregions of NSW: Sydney Basin, Riverina, Darling Riverine Plains, Cobar Peneplain, Mulga Lands and Broken Hill Complex (Fig. 2), habitats known to support some of the greatest diversity of mistletoe species globally (Vidal-Russell and Nickrent, 2008). Due to the inherently low abundance of mistletoes in this environment (Watson, 2009) and their patchy distribution (Rawsthorne et al., 2012), roadside sampling was undertaken to maximise encounter rates. Mistletoes are more abundant on roadsides due to increased run-off and other improvements in environmental conditions for hosts (Norton and Reid, 1997; Norton and Stafford Smith, 1999; Watson, 2009). Roadside sampling also maximises the diversity of habitats sampled while controlling for land use type. Upon arriving in a given bioregion, sampling commenced as soon as woody vegetation was observed, identifying the habitat type (after Keith, 2004) and carefully scrutinising all trees and shrubs within view of the road (from a slow-moving vehicle, either two or three observers scanning the vegetation on both sides of the road). Upon detecting the target mistletoe species, walking surveys commenced, thoroughly searching roadside vegetation until 25 mistletoe hosts were recorded. This number was chosen after initial analysis based on preliminary data from two mistletoe species determined that 25 records captured variation in host diversity both within and among habitat types within a bioregion (Milner, K. 2014. Honours thesis, cited earlier). Host species were identified

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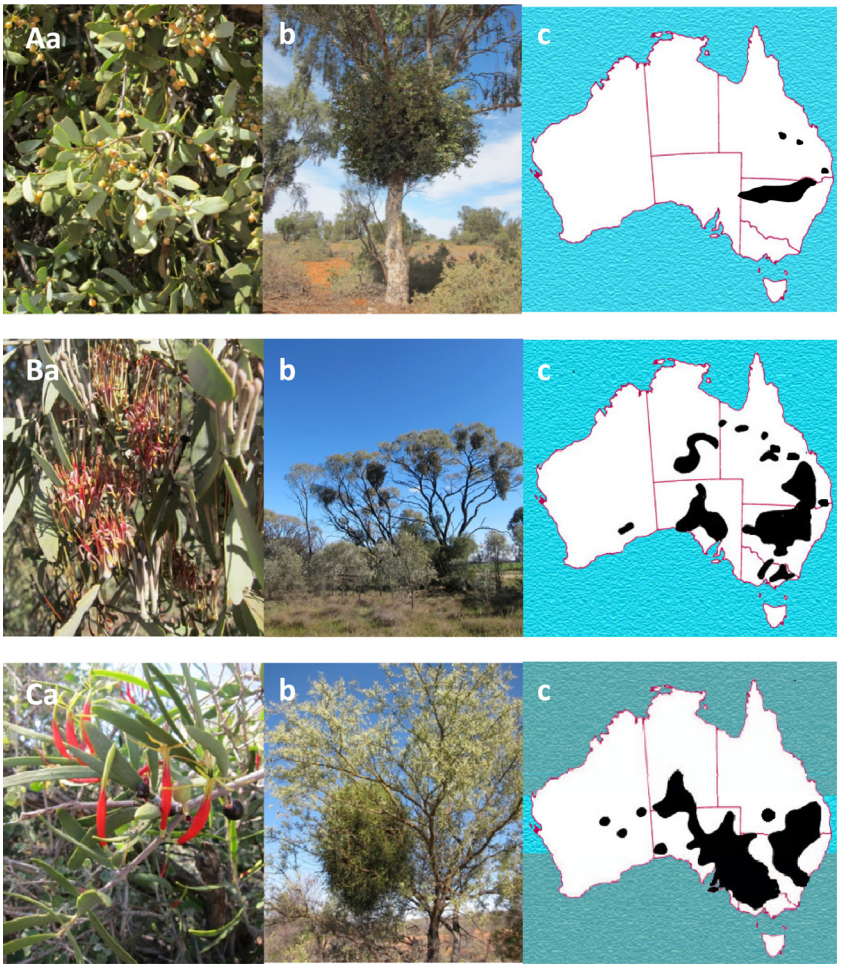

Fig. 1. Appearance and distribution of three study mistletoe species (after Watson, 2011). (A) *Amyema lucasii* in fruit. (a) Grey-green leaves are thick and taper towards the base; (b) it appears as an erect clump and often attaches to the main trunk; (c) the distribution is shown; (B) *Amyema quandang* in flower. (a) Red anthers and inner surface are clearly seen in contrast to the dull grey-green outer surface of the petals. (b) The appearance of *A. quandang* from afar is a teardrop shape and (c) the distribution. (C) *Lysiana exocarpi* in detail (a) with shiny red and yellow flowers in pairs, black fruit and succulent leaves. (b) Habit of *L. exocarpi* in a host tree, although a large specimen, shows the messy nature of the plant due to the woody branches, and (c) distribution. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to species level, excluding *Sennas* due to recent taxonomic changes in that taxon.

An iterative results-based stopping rule was used to determine when a bioregion had been sufficiently sampled. After sampling plots in a minimum of three different habitats (defined by Keith, 2004), predicted host richness was calculated using Abundance-based Coverage Estimator (hereafter ACE) (after Hortal et al., 2006; Basualdo, 2011), an estimator (Chao and Lee, 1992) that uses the proportion of individuals in rare host species that are not singletons (only one individual encountered per sampling effort) to calculate the predicted host species richness (S_{ace}) by:

$$S_{ace} = S_{abund} + \frac{S_{rare}}{C_{ace}} + \frac{F_1}{C_{ace}}\gamma^2_{ace} \quad (1)$$

where S_{abund} are abundant host species (>10 individuals); S_{rare} are rare host species (<10 individuals); C_{ace} is the sample coverage estimate and γ^2_{ace} is the estimate of the coefficient of variation of the F_1, the number of singletons (Colwell, 2013). Sample-based abundance data were analysed with the software program EstimateS v 9.1.0 (Colwell, 2013), in real time during sampling. To generate variance estimates free of any influence of sample order, a mean species accumulation curve was calculated from 100 randomisations (without replacement) of sampled host species data (Colwell et al., 2004). If observed host richness was <0.80 of predicted richness, sampling continued in the bioregion, with habitat types resampled. This level is typically where variance in completeness begins to decline and richness estimates stabilise (Watson, 2003). As each new sample plot was added, predictions were re-calculated until the 80% completeness threshold was met (after Lobo, 2008; Watson, 2010). ACE was selected a priori because it incorporates both richness and abundance data (Chao and Lee, 1992) and was used for all field-based determinations of sample completeness. In addition, seven other estimators were tested post hoc and evaluated on the basis of similarity of curve shapes through different data sets (after Melo and Froehlich, 2001) and sizes of standard deviations (after Walther and Moore, 2005). The results of the best-performing estimator/s were compared with those obtained by ACE.

Finally, observed host richness across multiple bioregions were used to predict host range across the distributional range of the parasite by pooling sample plots within a bioregion to give total abundance of each host species within a bioregion. Due to the lack of sample plots and bioregions for *A. lucasii*, data for all plots were entered individually at this step.

3. Results

A total of 1,075 trees were identified as hosts for the three mistletoe species within 10 habitat types across six bioregions of

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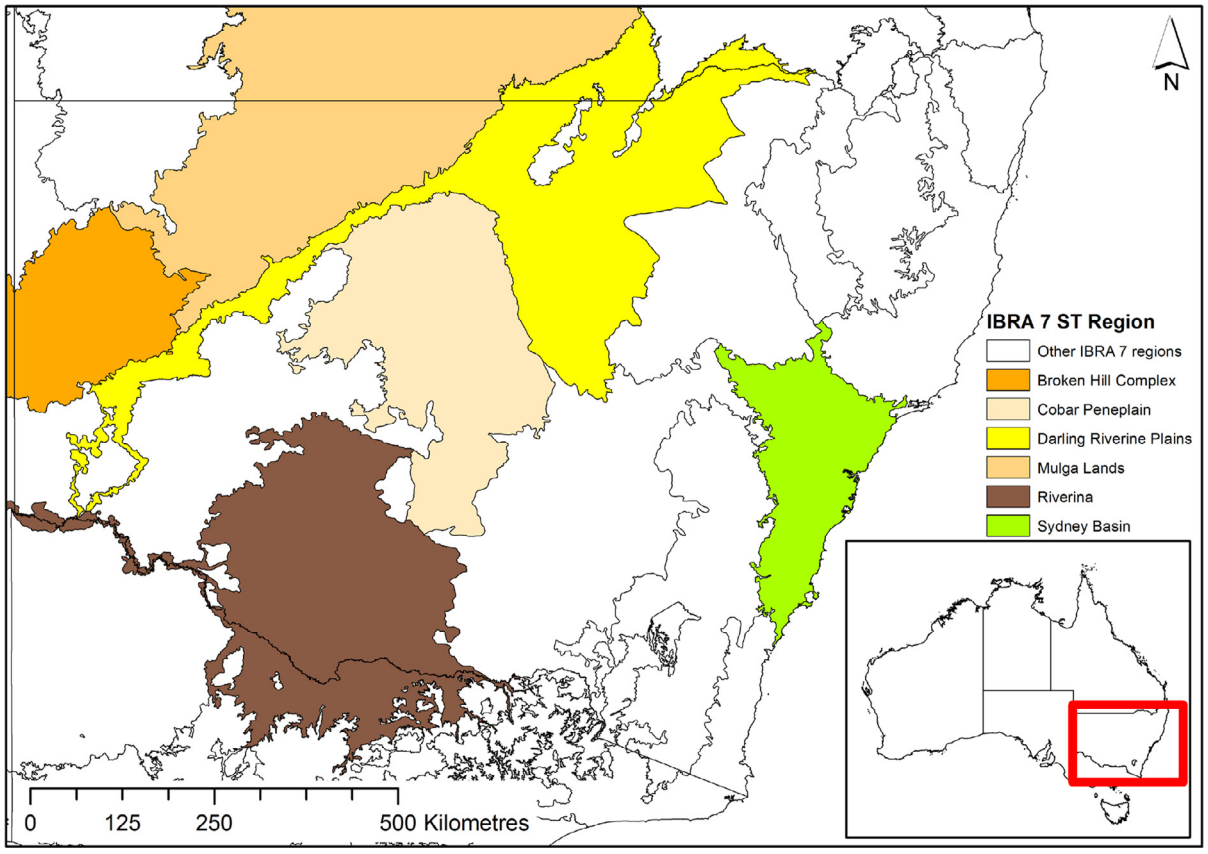

Fig. 2. Map of Australian bioregions. Sampling conducted in the New South Wales bioregions of Sydney Basin, Darling Riverine Plains, Cobar Peneplain, Riverina, Mulga Lands and Broken Hill Complex (as they appear from east to west). Based on IBRA 6.1 map (Department of the Environment, 2005).

NSW. The 625 host trees supporting *L. exocarpi* comprised 31 species in 13 genera from 12 families, including seven previously unrecorded host species (and one new family; Milner, K. 2014. Honours thesis cited earlier). The abundance data indicated that *Alectryon oleifolius* and *Eremophila sturtii* are important hosts for *L. exocarpi*. *Amyema quandang* was found on 325 host trees, comprising eight species in two genera from two families including two previously unrecorded host species (Milner, K. 2014. Honours thesis cited earlier). Abundance data indicate that primary hosts in the sampled regions are *Acacia pendula*, *A. homalophylla* and *A. aneura*. The 125 individual trees infected with *A. lucasii* were from a single species, *F. maculosa* (Rutaceae).

Of the four bioregions sampled for the generalist *L. exocarpi*, the stopping rule was satisfied after three and four sampling plots for the Sydney and Riverina bioregions, respectively, but terminated after six and 11 samples for the Mulga Lands and Broken Hill Complex bioregions, respectively, without reaching the 80% completeness threshold (using the ACE; Fig. 3A, C, E, G, Table 1).

Of the three bioregions sampled for the *A. quandang*, the stopping rule was satisfied after either three (Broken Hill Complex and Darling Riverine Plains bioregions) or six (Cobar Peneplain) sampling plots, reaching the 80% completeness threshold (using the ACE; Fig. 4, Table 1).

Mulga Lands was the only bioregion for which the specified three sampling effort plots were surveyed for strict specialist *A. lucasii*. An additional two plots were sampled in the Broken Hill Complex, but could not be tested because a minimum of three are required for EstimateS. The seemingly poor sampling attempt of only five plots was due to the fact that all plots yielded the same result: a single species was host for *A. lucasii* and all estimators predicted one host (Table 1).

3.1. Estimating host richness across a parasites distributional range

A total of 31 species were observed as hosts of the generalist *L. exocarpi* across the five bioregions sampled, of which 17 species were unique hosts and 10 were duplicates (i.e., only four host species were encountered more than twice). According to the estimators ACE and Bootstrap, the five bioregions sampled were enough to trigger the stopping rule, yielding completeness levels of 0.85 and 0.82, respectively (Fig. 5). These two estimators gave predicted host species (ACE S_{est} = 36, Bootstrap S_{est} = 38) at the lower end of values derived from other estimators (3555) and well short of the 106 native host species recorded for *L. exocarpi* across its entire distributional range (Table 2).

Sampling of *A. quandang* covered four bioregions and recorded the mistletoe as parasitising eight species, with a ratio of unique to duplicate species of 5:3. The predicted number of hosts over the geographic range of *A. quandang* varied from 9 to 16, well below the 53 species of known hosts (Table 2), despite ACE reaching 82% completeness (Fig. 5C). Jackknife (Jack1), the other estimator chosen, did not trigger the stopping rule as it reached only 68% (Fig. 5D). In this instance, Jack1 appears the better estimator as it suggests that sampling should continue in other bioregions.

Due to the under-sampling of *A. lucasii*, geographic range is based on sampling plot data rather than bioregional totals. All five transects found a single species (Fig. 5) and all estimators predicted a single host species.

4. Discussion

Host range is a critical life history trait of parasites, but comparative approaches to identify determinants of host range are

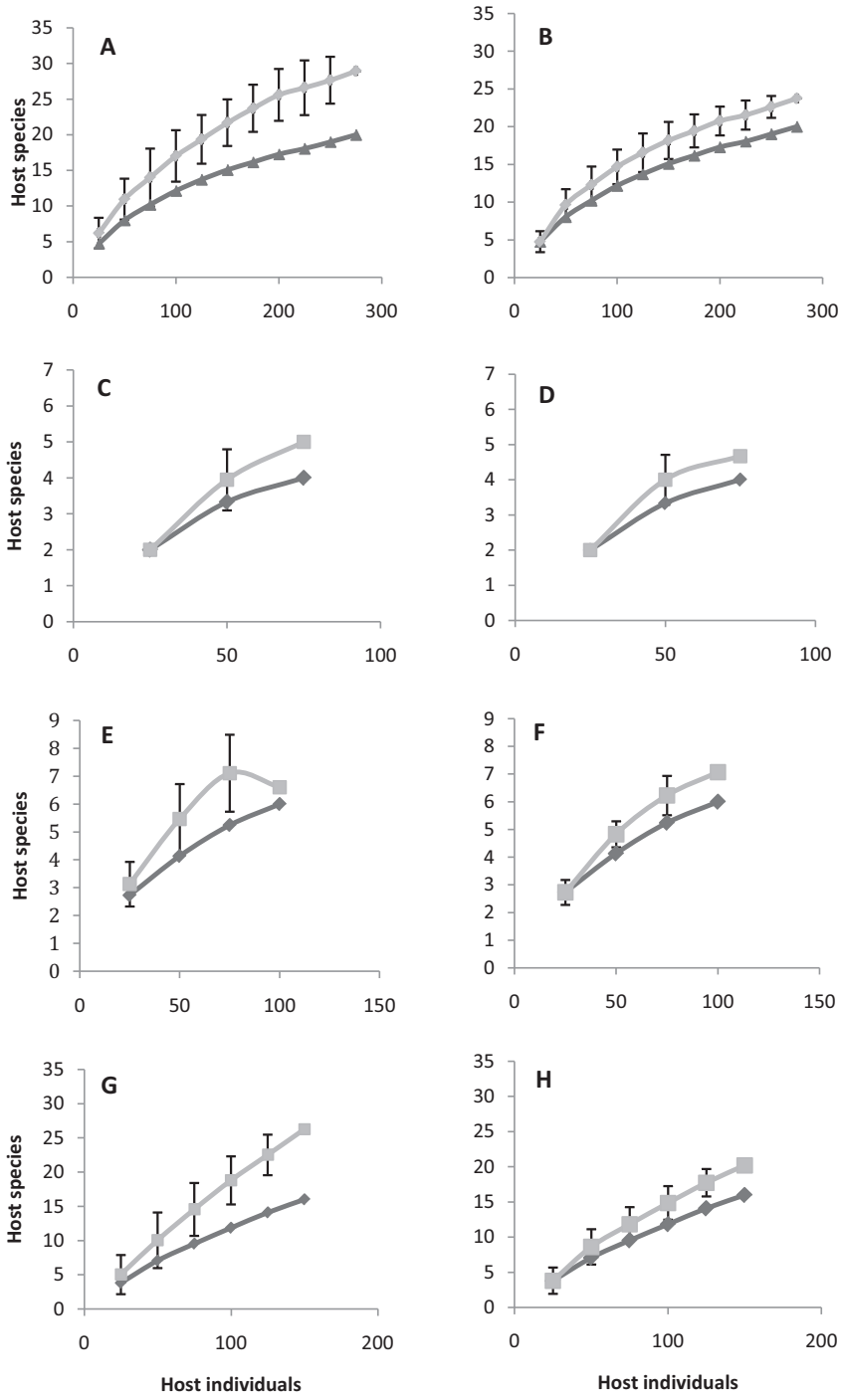

Fig. 3. Species accumulation curves for *Lysiana exocarpi* in bioregions of New South Wales, Australia. (A, B) Broken Hill Complex, (C, D) Sydney Basin, (E, F) Riverina and (G, H) Mulga Lands. The mean number of species observed is shown in black and estimated host species number in grey for (A, C, E, G) Abundance-based Coverage Estimator (ACE) and (B, D, F, H) Bootstrap estimators. Within the Mulga Lands bioregion, 16 host species were identified in six sampling plots but, given the high number of unique species (12), six samples were not enough to satisfy the stopping rule (ACE and Bootstrap reached 61% and 79% completeness, respectively). The S.D. of predicted host richness (denoted with error bars), however, continued to decline after the third point, suggesting sampling was near complete.

impeded by the confounding effects of sampling effort, with the number of known hosts increasing with more records. Here, we demonstrated a real-time method for estimating host range using a spatially-applied results-based stopping rule to determine how many sites to sample before arriving at a robust estimate of the overall number of species acting as hosts across the parasites distributional range. The iterative approach used to decide whether further sampling was required was successful in allocating effort where it was needed; those bioregions where the highest numbers of host species were observed required more sampling (711 sample plots) than bioregions where observed host species numbers were low (34 sample plots required to satisfy the stopping rule). In addition to being more efficient than either arbitrary or fixed allocation of effort, this targeted approach generated more ecologically-relevant and comparable estimates. Previously the standardised search had only been applied temporally to determine when to stop sampling a site so that estimates of species richness could be compared among sites (Watson, 2003, 2004, 2010;

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Table 1
Summary of observed and predicted host richness for three mistletoe species sampled across six bioregions in New South Wales, Australia.

	n	Hosts (o)	ACE[a]	Boot	Jack	U:D	Rare
Lysiana exocarpi							
Broken Hill	11	20	29 (0.69)	24 (0.83)		9:5	13
Sydney Basin	3	4	5 (0.80)	5 (0.80)		1:1	2
Riverina	4	6	7 (0.86)	7 (0.86)		1:1	1
Mulga Lands	6	16	26 (0.61)	20 (0.8)		12:2	8
Amyema quandang							
Broken Hill	3	2	2 (1.00)		2 (1.00)	0:0	0
Darling	3	2	2 (1.00)		3 (0.67)	1:0	0
Cobar	6	7	9 (0.78)		10 (0.70)	4:1	1
Amyema lucasii							
Mulga Lands	5	1	1	1	1		

n, the number of plots (each comprising 25 hosts); Hosts (o), the number of host species observed; ACE, predicted host richness using the Abundance-based Coverage Estimator; Boot, predicted host richness using the Bootstrap estimator with estimated completeness in parentheses; Jack, predicted host richness using the Jack1 estimator with estimated completeness in parentheses; U:D, the ratio of unique to duplicate hosts (those host species recorded only once or twice); Rare, those host species known from less than 1% of recorded host individuals.
All calculations were performed using EstimateS (Colwell, 2013).
[a] Estimated completeness derived by dividing predicted host richness by observed host richness (in parentheses).

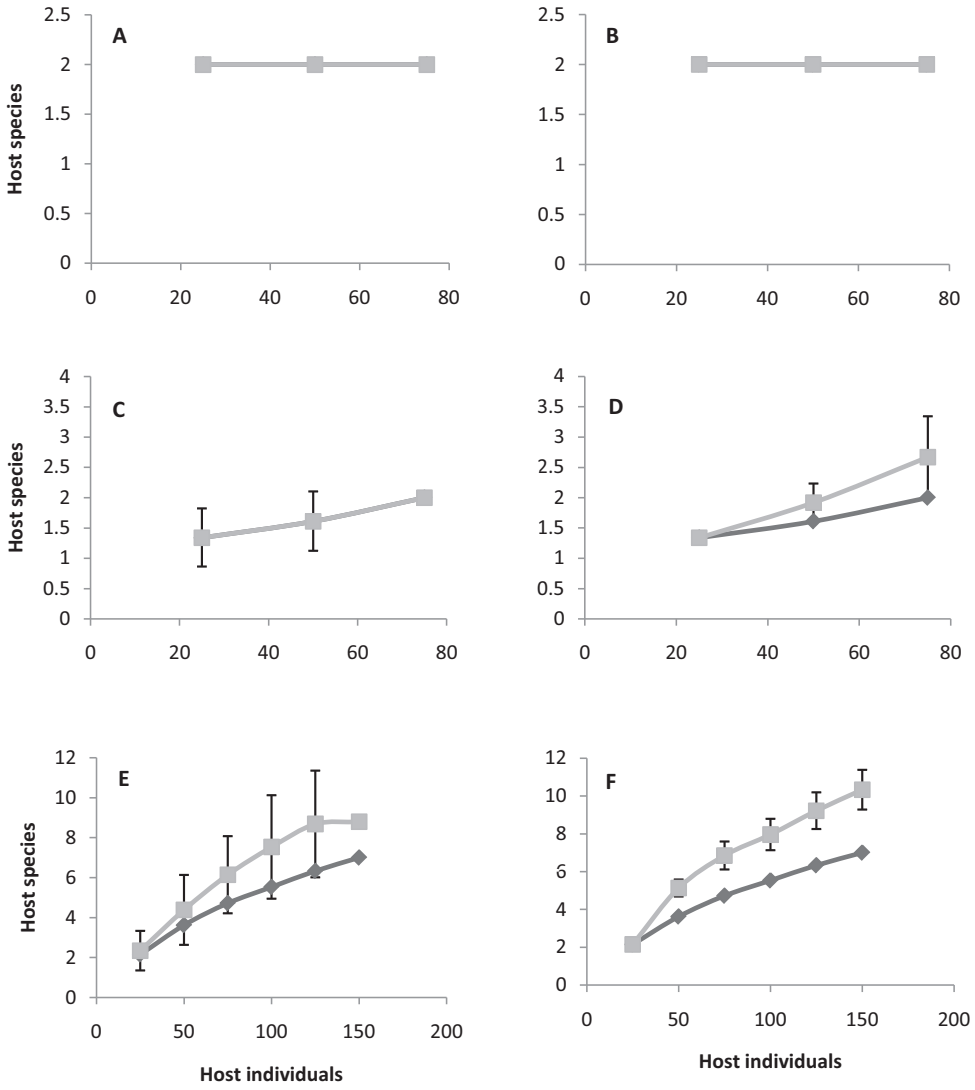

Fig. 4. Results for the three bioregions in New South Wales, Australia in which *Amyema quandang* was sampled are displayed for the estimators Abundance-based Coverage Estimator (ACE) and Jack1. Jack1 fits the species abundance curves of observed host species and shows consistently lower variance than other estimators. Species accumulation curves for *A. quandang* in (A, B) Broken Hill Complex, (C, D) Darling Riverine Plains, (E, F) Cobar Peneplain. The mean number of species observed is shown in black and estimated host species number in grey for (A, C, E) ACE and (B, D, F) Jack1 estimators. Error bars quantify the S.D. of predicted host species richness.

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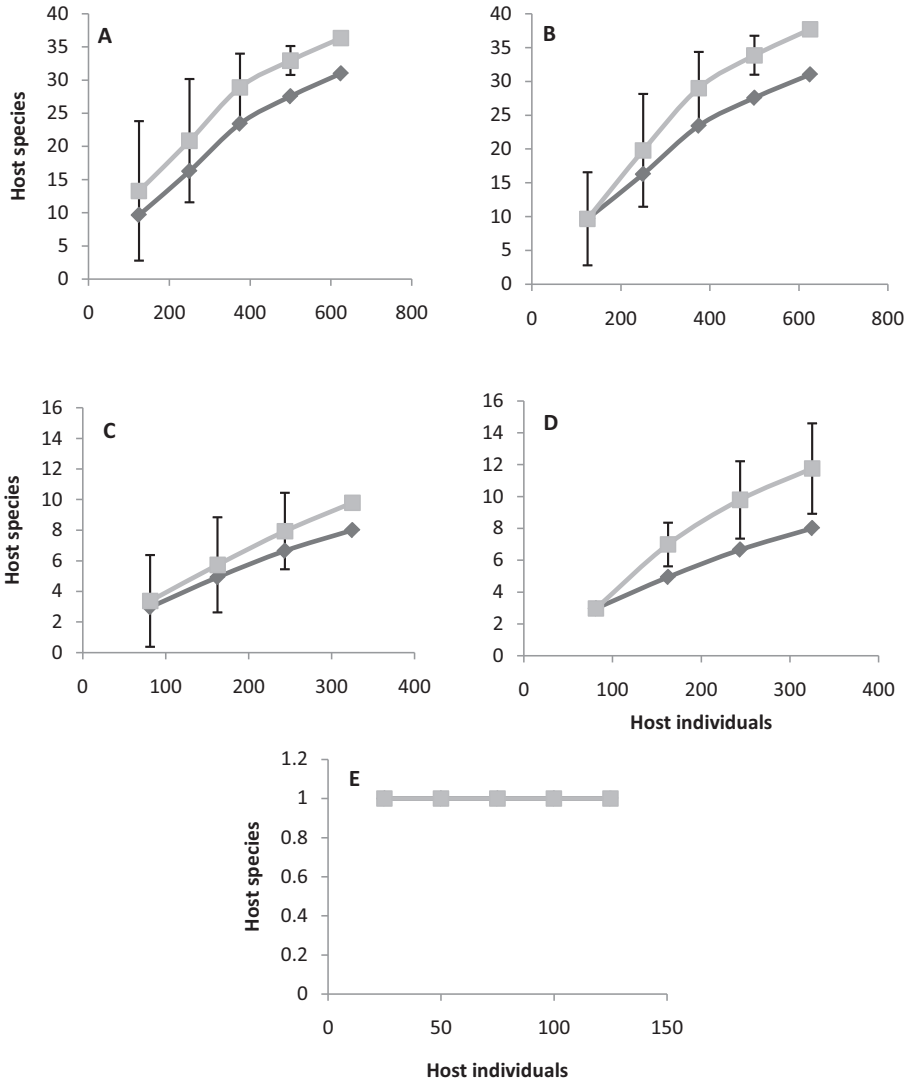

Fig. 5. Species accumulation curves for the three mistletoe species sampled across all bioregions in New South Wales, Australia. (A, B) *Lysiana exocarpi*, (C, D) *Amyema quandang* and (E) *Amyema lucasii*. The mean number of host species is denoted by grey diamonds, the predicted host species number is denoted by grey squares derived from Abundance-based Coverage Estimator (A, C, E) Bootstrap (B) Bootstrap and Jack1 (D) estimators. Error bars quantify the S.D. of predicted host species richness.

Table 2
Comparison of hosts recorded during this study with comprehensive host inventories derived from herbarium data.

Hosts	Families		Genera		Species	
	Observed	Total	Observed	Total	Observed	Total
Lysiana exocarpi	**11** + 1	22	**12** + 1	37	**24** + 7 (3555)	106
Amyema quandang	**2**	12	**2**	15	**6** + 2 (916)	53
Amyema lucasii	**1**	3	**1**	5	1 (1)	7

Total, previously recorded host families, genera and species for the three mistletoe species based on herbarium records summarised by Downey (1998; for native species only).
Observed, values relate to hosts we recorded, bold denoting the number of species common to our study and previous records + the number of previously unrecorded host families, genera and species. Values in parentheses represent the range of host species predicted using eight richness estimators.

Luck and Smallbone, 2011; Callaghan, 2012; Luck et al., 2013), but we demonstrated the approach is equally relevant when applied spatially to determine whether sampling additional sites is needed to obtain distribution-wide estimates of known completeness.

All three estimates of host range over the geographic distribution of the three mistletoes studied (*L. exocarpi* S_{est} = 3637, *A. quandang* S_{est} = 916 and *A. lucasii* S_{est} = 1) were well below the numbers of host species known from herbarium records (*L. exocarpi* S_{obs} = 114, *A. quandang* S_{obs} = 53 and *A. lucasii* S_{obs} = 7; Downey, 1998; Milner, K. 2014. Honours thesis cited earlier), differences that might reflect the estimators used and/or insufficient sampling. Sample coverage estimators are known to be conservative, giving estimates at the lower bound of the range (Chao and Lee, 1992; Hortal et al., 2006), and ACE and Chao 2 become sensitive to sample size when patchiness is high (Chazdon et al., 1998). Although sampling *L. exocarpi* hosts in two bioregions did not satisfy the

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stopping rule, moderate completeness levels were attained (6179% at the lowest; Table 1) and predicted host species richness of *L. exocarpi* appears to be approaching an asymptote as variance declines (Fig. 4).

The number of bioregions sampled is another consideration. Sample areathe extent of the samplinghas been noted as affecting estimated richness (McGeoch and Gaston, 2002). Here, sample area was low (small number of bioregions) compared to the geographic range (total number of bioregions) for which we predicted host range. Sampling a larger number of bioregions for the three study mistletoes and/or sampling other mistletoe species across their geographic range would easily address whether a sampling issue is responsible for low estimates of host range over geographic distribution. Both Jackknife and Bootstrap estimators underestimate the number of species if there is low sample size or a large number of rare species (Smith and van Belle, 1984), especially when extrapolating from smaller-scale bioregional estimates to predict the host range across the entire geographic range (see Chazdon et al., 1998).

In identifying hosts, new mistletoe-host combinations were recorded for *L. exocarpi* and *A. quandang*, supporting the idea that a host species list may never be complete (Downey, 2004; Grenfell and Burns, 2009). These new records also highlight another limitation of simple host liststhe implicit assumption that all host species are equally important to a parasite (Poulin and Mouillot, 2005). Some mistletoe-host combinations are rare, recorded <10 times; similar values have been used to note infrequent hosts (Reid and Lange, 1988). Other host species are used more frequently and could be considered the primary or ecologically meaningful hosts. Rather than inventories or simplistic, arbitrary distinctions between specialist and generalist, we subscribe to an emerging view of host-specialisation as a spectrum. Thus, specialists may have a primary host/s and a number of less common/sporadic host species (Norton and Carpenter, 1998; Giorgi et al., 2004) whereas generalist parasites can show preferences for some hosts but the suite of hosts they can use is far greater (Norton and Carpenter, 1998). Many of the species identified as hosts for *A. quandang* and *L. exocarpi* in this study have previously been noted as common host species (Barlow, 1984; Yan, 1990, 1993; Keith, 2004; Barea and Herrera, 2009; Watson, 2011), suggesting that the approach we developed here generates robust estimates of the number of primary hosts, enabling the position of these mistletoe species along the spectrum of host specialisation to be quantified and compared.

Applying this idea to our data, we suggest that our estimates are a fair representation of the actual host range over the geographic distribution for the mistletoes studied. That our estimates are lower than the inventory based on herbarium records may relate to known biases associated with herbarium data (e.g., collector bias, GarcillÃn and Ezcurra, 2011). Optimal sampling reduces sampling bias, leading to exclusion of accidental hosts from oversampling (Poulin, 1992; Walther et al., 1995). Previous studies of sampling bias have not considered whether host range is revised up or down when controlling for sampling effort (Gregory, 1990; Poulin, 1992; Walther et al., 1995; Grenfell and Burns, 2009) so results here cannot be compared with previous studies. Revising host range down (i.e., interpreting some previously recorded host species listed as an artefact of oversampling) is most likely in this case, considering that few widespread native host species were missed when sampling.

Looking beyond the absolute number of species, consider the ecological information contained in the assemblage of species identified as hosts for the three mistletoes we studied. *Lysiana exocarpi* is known from 114 hosts (106 native species and eight exotics), compared with 53 species for *A. quandang*. Our estimates of host range parallel these figures, with approximately double the number of species predicted for *L. exocarpi*. In terms of higher taxonomic units, we recorded *L. exocarpi* on 13 host genera from 12 families (Table 2). Although representing approximately one-third of the number of genera and half the number of families known from herbarium records, our data support the characterisation of this species as a generalist. Indeed, seven of the 31 species we identified as hosts represent previously unrecorded species (one of which represents a new family). Our results for *A. quandang* paint a different picture. In contrast to herbarium data (53 hosts, 15 genera, 12 families), we recorded eight hosts from two genera in two families, seven of which were in the genus *Acacia* (including two previously unrecorded mistletoe/host combinations). Our data strongly support the characterisation of this species as a specialist on the genus *Acacia*, an inference that would be difficult to support based on the host number generated from herbarium records. Similarly, our findings for *A. lucasii* conform to previous work suggesting this is one of the most specialised of Australian mistletoes, occurring exclusively on *F. maculosa* (Barlow, 1984; Cunningham et al., 1992). Although recorded on six other host species in four other genera from two other families (Table 2), we suggest this herbarium data is misleading and *A. lucasii* (at least in the western half of its distributional range) is best considered a species-specific mistletoe.

With appropriate modification of the protocol and knowledge of the system under study, we believe the standardised search can be applied to other types of parasites to effectively estimate host spectrum. This method would be readily transferrable to ectoparasites (e.g., mites, ticks and lice of birds and mammals (Krasnov et al., 2004, 2005)), parasites with direct lifecycles (e.g., mites on bats, Giorgi et al., 2004; monogeneans and copepods on fish, Poulin, 1992). It also could be applied to parasites with complex life cycles where one stage can be studied in isolation (e.g., birds as the definitive hosts of trematodes, Lafferty and Morris, 1996) and to endoparasites where infection is apparent externally (e.g., cysts on fish infected with microsporidian parasites, Ward, 2005). For more inconspicuous parasites, sampling would necessarily be more effort-intensive than with conspicuous parasites. To keep sampling efficient it would be especially important to design targeted sampling; for example, sample areas where known hosts occur. Due to the rarity of parasites in the environment it may be more efficient to target known peaks in parasite prevalence (Cosgrove et al., 2008). Knowledge of the time/season when prevalence peaks (e.g. the highest prevalence of haematozoan parasites in woodpeckers is during summer, Schrader et al., 2003) or the behaviour of the host that leads to the peak in prevalence (e.g., during breeding season, Hatchwell et al., 2000) can be used to optimise sampling.

Currently, host ranges for parasites are estimated (i) based on incomplete sampling, often yielding spurious results (Norton and Carpenter, 1998; Grenfell and Burns, 2009); (ii) based on exhaustive sampling, but with oversampling clouding ecological relevance (Poulin, 1992) especially when all host-parasite combinations are treated as equally important (Poulin and Mouillot, 2005; Poulin et al., 2011); or (iii) using sufficient sampling but at great cost in terms of sampling efficiency (Walther et al., 1995; Peterson and Slade, 1998). Adapting the standardised search approach to other parasite systems overcomes all of these issues: (i) sampling continues until required completeness is obtained, (ii) primary or preferred hosts dominate the dataset, making it ecologically meaningful and (iii) the optimised sampling effort is highly efficient. Further, because sampling effort is standardised by a stopping rule based on sampling completeness rather than a pre-determined or arbitrary sampling effort, the results for different parasites can be directly compared, generating more widely applicable information on relative host ranges.

Please cite this article in press as: Watson, D.M., et al. Novel application of species richness estimators to predict the host range of parasites. Int. J. Parasitol. (2016), http://dx.doi.org/10.1016/j.ijpara.2016.10.001

Acknowledgements

We are grateful to field work assistants Karen Avery, Adrian Boyd, Melinda Cook, Wendy Scott and David Roach, and to Maggie Watson for logistical support during fieldwork and considered input on an earlier version of this manuscript.

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