

The use of incidence-based species richness estimators, species accumulation curves and similarity measures to appraise ethnobotanical inventories from South Africa

Vivienne L. Williams · Edward T. F. Witkowski · Kevin Balkwill

Received: 2 December 2003 / Accepted: 20 February 2006
© Springer Science+Business Media B.V. 2006

Abstract The incorporation of suitable quantitative methods into ethnobotanical studies enhances the value of the research and the interpretation of the results. Prediction of sample species richness and the use of species accumulation functions have been addressed little in applied ethnobotany. In this paper, incidence-based species richness estimators, species accumulation curves and similarity measures are used to compare and predict species richness, evaluate sampling effort and compare the similarity of species inventories for ethnobotanical data sets derived from the trade in traditional medicine in Johannesburg and Mpumalanga, South Africa. *EstimateS* was used to compute estimators of species richness (e.g. Jackknife), rarefaction curves, species accumulation curves and complementarity. Results showed that while the Michaelis–Menten Means estimator appeared to be the best estimator because the curve approached a horizontal asymptote, it was not able to accurately predict species richness for one of the data sets when two of its subsamples were individually tested. Instead, the first-order Jackknife estimator best approximated the known richness.

Keywords Traditional medicine trade · Quantitative ethnobotany · Species accumulation curves · Rarefaction · Richness estimation · Complementarity · Sampling effort · *EstimateS*

Introduction

Species richness, or the number of species in a sample of a specified size, is an instantly comprehensible expression of species diversity (Magurran 1988). Biologists often need to know how many species there are within an area or, they need to compare the number of species found in different areas (Colwell et al. 2004).

V. L. Williams (✉) · E. T. F. Witkowski · K. Balkwill
School of Animal, Plant and Environmental Sciences, University of the Witwatersrand,
Private Bag 3, Wits 2050, South Africa
e-mail: vivwill@planetac.co.za

Complete enumeration of species richness within an extensive study area is, however, generally not feasible and consequently a number of different methods for estimating total species richness from a sample have been devised (Chiarucci et al. 2003). Traditionally, species accumulation functions have been used for plotting species versus sampling effort to estimate visually whether a curve has asymptotically approached some ceiling (and hence whether sampling has been sufficient), as well as estimate the total richness of a site (Soberón and Llorente 1993; Gotelli and Entsminger 2001). Colwell and Coddington (1995), Gotelli and Colwell (2001) and Colwell et al. (2004) have explored the measurement, estimation and comparison of species richness through extrapolation and the use of taxon sampling curves, including assigning confidence intervals to the estimates. While the methods for estimating species richness have been applied to the approximate assessment of species richness in faunal and floral groupings at comparative sites (e.g. by Bunge and Fitzpatrick 1993; Soberón and Llorente 1993; Colwell and Coddington 1995; Toti et al. 2000; Gotelli and Colwell 2001; Chiarucci et al. 2003; Sørensen 2004), the benefits of such an approach to applied quantitative ethnobotany have yet to be fully explored.

Ethnobotanical surveys are often the first step in identifying useful species that are a resource management priority (Cunningham 2001). A challenge for ethnobotanical studies has been in knowing how complete the plant inventories are, i.e. how many more species would have been documented with increased sampling effort? Furthermore, how does a researcher objectively decide on the level of sampling effort required to satisfactorily complete a survey or draw conclusions on the adequacy of the effort already executed? In two of the few studies using species accumulation functions for ethnobotanical data, Begossi (1996) and Hanazaki et al. (2000) used rarefaction curves to evaluate sampling effort and explore differences in plant use per category of user (e.g. age and gender) within different communities, mainly in South America. These methods have the potential to be broadened to other ethnobotanical studies and resource inventories to include comparisons of species richness between sites, as well as estimate the number of species expected had a complete census of all the plants used/traded been possible. Plant inventories for ethnobotanical studies are usually incidence-based (i.e. presence or absence). The aim of this paper is to assess the usefulness of incidence-based species accumulation curves, species richness estimators and similarity measures with data collected from the medicinal plant trade in the Witwatersrand region (including Johannesburg), South Africa.

Species accumulation curves

A *species accumulation curve* is the graph of the cumulative number of observed species as a function of some measure of sampling effort (Colwell et al. 2004). If the species are randomly and sequentially recorded one after another within a sampling area, then the resulting accumulation curves are *individual-based*. If, however, the survey area is subdivided into smaller sampling units (such as quadrats) and the total number of species is accumulated as a result of successively sampling additional quadrats, then the accumulation curves are *sample-based* (Gotelli and Colwell 2001). Sequential or successive pooling of the individuals or samples will not, however, produce a smoothed curve and the shape very much depends on the sample order.

Rarefaction is used to produce a smoothed curve that is the statistical expectation of the corresponding accumulation curve (Gotelli and Colwell 2001; Colwell et al. 2004). Rarefying a sample estimates its expected species richness at different values of n samples or N individuals from the pooled total species richness after randomizing the sample order. Rarefaction curves can be individual- or sample-based, depending on the sampling methodology (Colwell 2001). In ethnobotany, the quadrat as a sampling unit would be replaced with a more appropriate sampling entity such as herbalists' shop, street traders' stall, or an informant within a community. Comparisons of the species accumulation curves with the rarefaction curves can be used to assess the homogeneity of the samples. In addition, the 95% confidence intervals of the rarefaction curves calculated by *EstimateS* (Colwell 2005) can be used to determine whether species richness for data sets is significantly different.

Estimating species richness

There are three approaches to estimate species richness from samples, namely (1) fitting parametric models of relative abundance (e.g. log normal distribution), and using the shape of the species abundance distribution to deduce the total species richness, (2) non-parametric estimators, and (3) extrapolation of species accumulation or species-area curves (Magurran 2004).

Parametric methods

Two parametric models of relative abundance with the greatest potential for estimating species richness are the log normal and log series distributions (Magurran 2004). When species fit a log normal model, the relative abundance of the data takes the form of a bell-shaped normal distribution (Krebs 1989). It is possible to estimate the theoretical number of species (S^*) in the community/assembly by extrapolating the bell-shaped curve beyond the class of minimal abundance (i.e. over the 'hidden' portion of the curve to the left of the 'veil line') and measuring the area under the curve (Krebs 1989; Colwell and Coddington 1995). Most log normal distributions are, however, of the truncated variety (Magurran 1988, 2004), i.e. part of the bell-shaped curve is obscured, and the truncation point is at the veil line. Pielou (1975) devised a method for fitting a truncated log normal distribution that assumes that the position of the veil line can be recognized, and that the area under the remaining part of the curve can be used to estimate S^* (Magurran 1988).

Data that fit a log series distribution show a 'hollow' curve. In other words, comparatively few species are common and comparatively large numbers of species are rare (or, infrequently recorded). Additionally, the modal class is always the singletons no matter how large the sample (Colwell and Coddington 1995). The estimate of species richness is always non-asymptotic; hence, the number of estimated species will increase as the number of individuals (N) increases (Magurran 2004).

Non-parametric methods

Non-parametric methods for estimating species richness from samples were adapted from mark-recapture applications for estimating population size. They also

require no assumptions about community structure (Colwell and Coddington 1995). *EstimateS* by Colwell (2005) computes seven non-parametric estimators of species richness. The estimators are homologous in that richness is estimated from the preponderance of infrequently occurring species in each sample, i.e. the higher the proportional abundance of rare species, the greater the probability of encountering more new species with increased sampling effort. The method for determining the best estimator involves visually estimating whether a plot of the estimated number of species for each successively accumulated sampling unit reaches a horizontal asymptote and reduces bias, following which conclusions are drawn regarding the adequacy of the size of the data set and the performance of the estimator. Sampling, or a census, is essentially complete if all species are observed “multiple” times or are “not rare” (Colwell and Coddington 1995). It is essential to note that richness estimators do not extrapolate beyond the last sample to estimate richness at an asymptote (Soberón and Llorente 1993). Instead, richness estimators predict richness, including species not discovered in the sample, from the proportional abundances of species within the total sample (Soberón and Llorente 1993; Colwell 2005).

Extrapolating species accumulation curves

Species accumulation curves illustrate the rate at which new species are found, but the total species richness of a site will not be directly revealed by the curves if sampling is not thorough (Magurran 2004). If sampling is incomplete, then the curves will show an upward trend illustrating the extent to which sampling effort might have to be increased to accumulate more species. The curves can also be used as a means of estimating species richness, most commonly by fitting functions such as the asymptotic Michaelis-Menten algorithm (Colwell et al. 2004), or non-asymptotic estimators such as log-linear models (Colwell and Coddington 1995) (not investigated in this paper because the non-parametric estimators are reported to perform better). Extrapolation of the curves is also possible, but there is no firm consensus on how to do so (Magurran 2004). Colwell et al. (2004) have explored the challenges of extrapolating species accumulation curves to estimate species richness, and the means to do so.

Complimentarity and similarity

Complimentarity is the measure of biotic distinctness or dissimilarity between species inventories that were compiled at sites located randomly within a habitat mosaic or along a gradient (Colwell and Coddington 1995). The greater the degree of distinctiveness, the more complimentary the two sites are, and hence the higher the combined species richness is likely to be between the sites. The measure captures the sense that species richness can be interpolated between site gradients depending on the degree of distinctiveness. Undersampled sites can, however, result in complimentarity and species richness being overestimated because the distinctness and the number of species shared between samples would be lower. In ethnobotanical studies, complimentarity can be used to measure the dissimilarity of species inventories between different markets and/or user groups and thereafter be used to interpolate the number of species that might be traded or in use between certain

sites or user groups. By contrast, *similarity* is the degree to which the species composition of samples is alike. The most widely adopted measures are the classic Jaccard and Sørensen indices for incidence-based data, and the Bray–Curtis (equal to the ‘Sørensen quantitative’ index) and Morisita-Horn indices for abundance-based data (Magurran 1988; Colwell 2005).

Study area

This case study is focused on the Gauteng Province of South Africa, and specifically the region called the Witwatersrand, which includes Johannesburg in its centre (Fig. 1). The Witwatersrand is an extensively urbanized axis of approximately 100×40 km, and has a large urban Black population with an active trade in traditional plant medicines. Johannesburg forms the hub of the regional informal plant trade, mainly because of the concentration of the labour force and the strong links of that labour force with rural communities (especially to the province of KwaZulu-Natal to the south-east) and traditional activities, including the utilization of traditional medicine. There were estimated to be 18,000 traditional healers in Soweto, a Johannesburg township, in the mid-1990s.

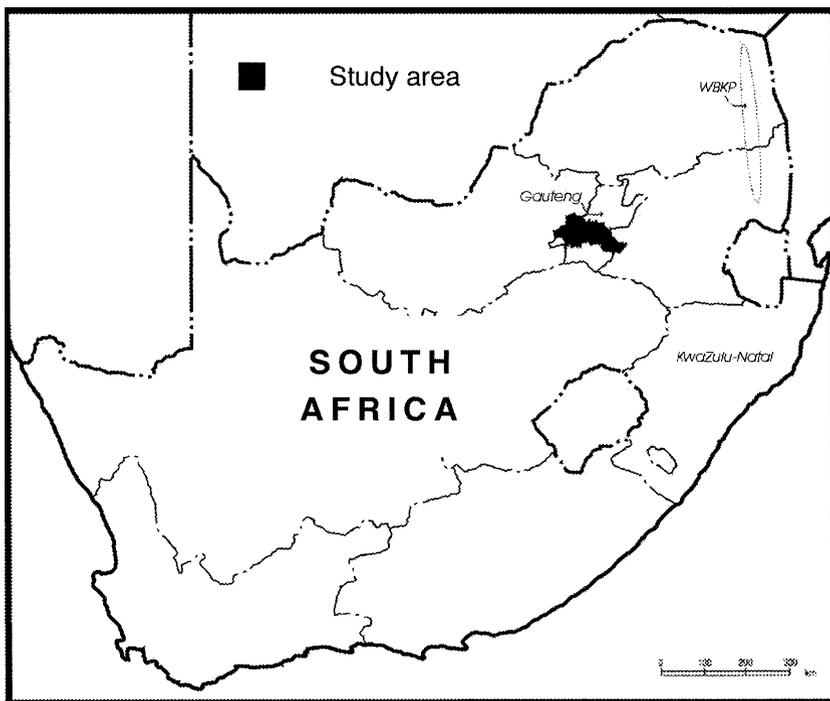


Fig. 1 The Witwatersrand study area (in black) within the province of Gauteng, South Africa. The Witwatersrand includes the metropolis of Johannesburg. One-hundred and fifty traders of traditional medicine were interviewed in the region between 1994 and 2001. ‘WBKP’ represents a region within which a survey of 17 traders was conducted by Botha (2001) on the western boundary of the Kruger National Park. The province of KwaZulu-Natal is the harvesting source of most plants sold in the Witwatersrand

The trade in medicinal plants is divided into two sectors, namely formal business represented by ‘*muti*’ shops or herbal pharmacies, and informal markets represented by street traders. In 1994, there were more than 200 *muti* shops on the Witwatersrand (Williams et al. 1997), the majority of which were owned by Black traditional healers (52%) and Indian merchants (25%). A study conducted in 2001 revealed there to be more than 160 street traders in a market called ‘Faraday’ (Williams 2003). Ninety-seven percent of the street traders were migrants to Gauteng, of which 90% regarded KwaZulu-Natal as ‘home’ (Williams 2003). For the most part, the trade of medicinal plants in Johannesburg meets the needs of the Zulu sector of the population, despite the multicultural nature of the metropolis (Williams et al. 1997). Customers to this market are primarily traditional healers from the region buying plants to restock their home-based healing practices.

Methods

The species accumulation functions were reviewed using seven sets of ethnobotanical data derived from inventories compiled from three surveys of medicinal plants in trade. The studies were conducted with formal *muti* shop traders and informal street traders of traditional medicine. The first survey in 1994 was for 50 *muti* shops on the Witwatersrand (‘*All shops*’) (Williams et al. 2000). This sample was subdivided into three smaller subsamples based on the ethnicity of the shop owner, namely Black ($n = 28$ shops), Indian ($n = 20$) and White ($n = 2$); data set was not included in the analyses due to its size). The second survey was conducted in 2001 with 100 street traders in the Faraday street market in Johannesburg (Williams 2003). The third survey, conducted by Botha (2001) with 17 informal street traders, inventoried plants traded on the western boundary of the Kruger National Park (‘*WBKP*’), Mpumalanga, South Africa. The seventh data set is a combination of all the ethnosppecies inventoried during the 1994 and 2001 Witwatersrand studies and is called ‘*WRand Total*’ ($n = 150$). This data set was only used in complementarity and similarity analyses because of the variations in sampling technique and the number of individuals per sample. All inventories recorded the presence of plants in a shop or at a stall, hence the data are incidence-based (qualitative) not abundance-based (quantitative).

‘*Ethnospecies*’ was used as a proxy for species during the analyses (except where otherwise specified). The term ‘ethnospecies’ (Hanazaki et al. 2000) takes into account the folk or common names given to one or several species quoted during resource inventory interviews. The ethnospecies name ‘*iMphepho*’, for example, applies to at least six species of *Helichrysum*, but it was not possible to ascertain which of the six the traders sold when the survey was conducted. Ethnospecies names are best used where uncertainty exists as to the correct identification of a species. Therefore, rather than duplicate the incidence of species occurrences, the data were quantified based on the number and frequency of occurrence of ethnospecies sold by the traders. Where more than one ethnospecies name applied to a single species, only one name was used during the analyses. Later in the paper, the ratio of ethnospecies to number of species is calculated for the specific surveys in order to extrapolate the estimated number of ethnospecies to actual species (e.g. Table 3). In the Faraday study, for example, 100 ethnospecies names \approx 136 plant species.

Public-domain software called *EstimateS* (Version 7.5, Colwell 2005) was used to calculate and evaluate the performance of six species richness estimators appropriate

for incidence-based data, namely: ICE, Chao 2, first order jackknife, second-order jackknife, bootstrap and Michaelis–Menten Means (Table 1). The Michaelis–Menten Runs estimator was considered, but eventually excluded from the analyses because of the very high and erratic richness estimates at the beginning of the curve. *EstimateS* was also used to compute the expected species accumulation curves (sample-based rarefaction) and 95% confidence intervals. The sample order was randomized 100 times to compute the mean estimator and expected species richness for each sample accumulation level. In doing so, a smoothed curve was generated. Species richness was also estimated using the log normal and log series parametric models of relative abundance. The formulae in Magurran (1988), Krebs (1989) and Ludwig and Reynolds (1988) were used to fit the data to the distributions.

Percentage complementarity and the classic Jaccard and Sørensen indices of similarity for incidence-based data were used to compare the distinctiveness of the data sets and calculate the combined species richness for two data sets. Complementarity was calculated using the formula in Colwell and Coddington (1995) and variables computed by *EstimateS*. The Jaccard and Sørensen indices were also computed by *EstimateS*. Complementarity coefficients vary from 0 (least complementary and most identical) to 1 (most complimentary and least identical), whereas similarity measures range from 0 (least similar) to 1 (most similar). Alternatively, the measures are expressed as a percentage.

Results and discussion

Species accumulation and rarefaction curves

Non-randomized species accumulation and sample-based rarefaction curves were plotted for species inventoried in the Faraday Street market and *muti* shops (Fig. 2). The 95% confidence intervals for the rarefaction curves were also plotted. The

Table 1 Brief descriptions of the species richness estimators used that are computed by *EstimateS* (Version 7.5) (From Colwell and Coddington 1995; Colwell 2005)

Name	Richness estimators	Description
<i>Non-parametric estimators</i>		
ICE	Incidence-based coverage estimator	Based on species found in 10 or fewer sampling units
Chao 2	Chao 2 richness estimator	Incidence-based; based on the presence of singletons and doubletons
Jack 1	First-order Jackknife richness estimator	Based on the number of species occurring in only 1 sample
Jack 2	Second-order Jackknife richness estimator	Based on the number of species that occur in only 1 sample, as well as the number that occur in exactly 2 samples
Bootstrap	Bootstrap richness estimator	Based on the proportion of the samples containing each samples
<i>Asymptotic extrapolation of the species accumulation curve</i>		
MMMean	Michaelis–Menten Mean richness estimator	Computes estimates once for each sample pooling level

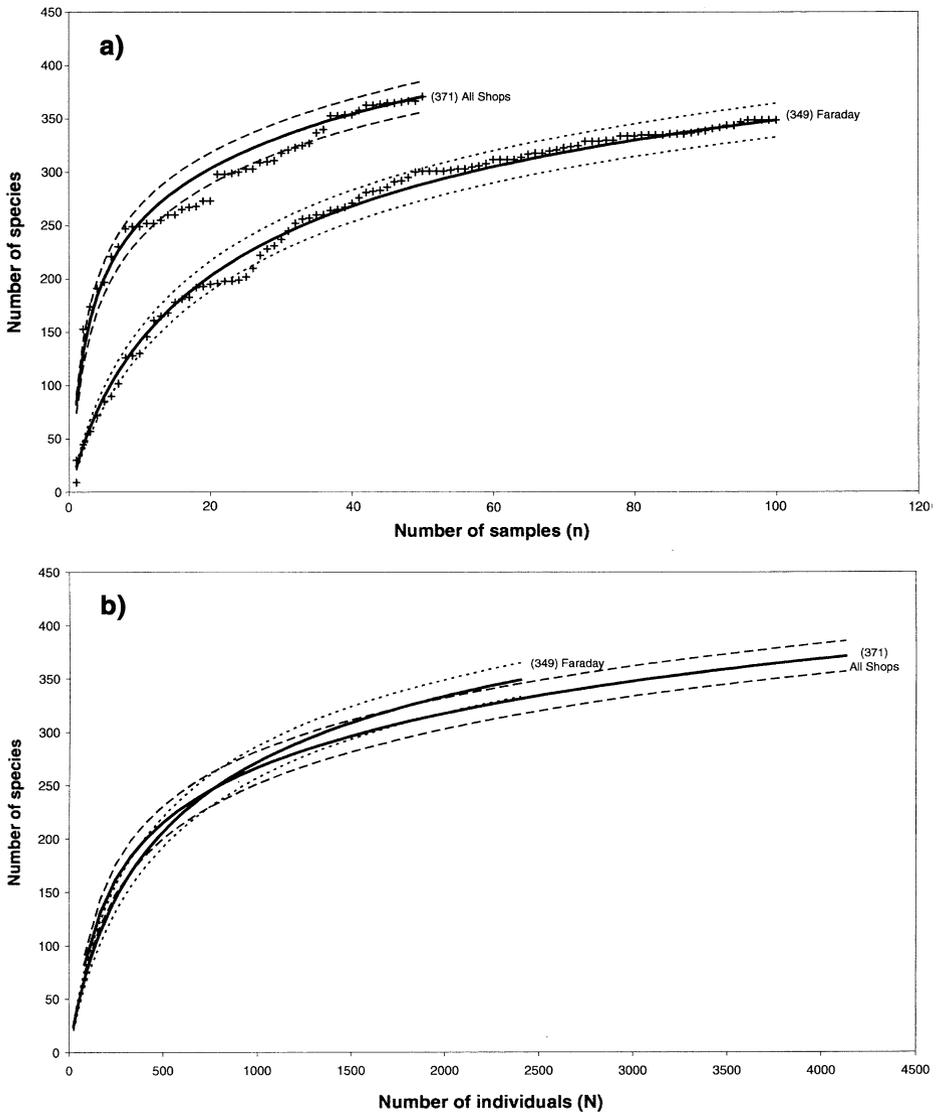


Fig. 2 Comparing sample-based rarefaction curves (solid-lines), 95% confidence intervals (dashed lines) and the non-randomized species accumulation curves (shown as + in 2a) for species recorded in the stalls of 100 street traders in the Faraday market in 2001 and in 50 *muti* shops in 1994. The x-axes are scaled (a) by number of samples, and (b) by number of individuals

x-axes are scaled by accumulated number of samples (i.e. the number of stalls or shops inventoried) (Fig. 2a) and, the number of individual plants accumulated as the samples were pooled (Fig. 2b). The graphs differ in that the number of stalls sampled in Faraday was twice that of the number of shops sampled, however the mean number of ethnospecies sold per shop trader was approximately 3.5 times the number of ethnospecies sold per stall in the Faraday market (means and standard deviation equal 83 ± 46 and 24 ± 12 ethnospecies per trader for *All shops* and

Faraday respectively). Numerical species richness per trader is thus greater for the shop traders, and the initial steep gradient of the curve shows that species are accumulated more rapidly for comparatively smaller sample numbers. For example, the number of ethnospecies expected by the rarefaction curve after 25 samples is 320 for *All shops* and 224 for *Faraday*—a difference of 96 ethnospecies (Fig. 2a). Figure 2a also shows that *multi* shops have consistently higher number of ethnospecies for similar levels of sampling effort compared to stalls at the Faraday market.

In ecology, Fig. 2a would represent a comparison between the densities of species for two data sets (i.e. the number of species per sampling unit), and Fig. 2b a comparison of species richness because the effect of density is removed when the individuals are pooled. When comparing sample-based rarefaction curves and 95% confidence intervals for two data sets derived from tropical rain forest saplings, Colwell et al. (2004) concluded that where the confidence intervals don't overlap, the differences in density and/or species richness are not significant at $P < 0.05$. If one uses this line of reasoning when comparing the differences in the mean number of ethnospecies per sample (Fig. 2a), then the differences are ostensibly significant because the confidence intervals do not overlap for the two data sets. However, when the x -axis is rescaled by individuals (Fig. 2b), then species richness for the two data sets is not significantly different. Gotelli and Colwell (2001) recommend that when comparing and estimating species richness between data sets and sample sites, the sample-based rarefaction curves are rescaled by individuals to adjust for the differing densities of individuals (Colwell et al. 2004).

The order in which the samples (or individuals) are included in a species accumulation curve influences its overall shape (Magurran 2004). Despite the non-randomized species accumulation curve not being smoothed (represented by ++ in Fig. 2a), the samples are relatively homogenous and there is little variation in the number of ethnospecies per sample—as indicated by how closely the species accumulation curve approximates the rarefaction curve (especially for *Faraday* data). The traders therefore consistently sell similar numbers of species.

The ability of a species accumulation curve to reach an asymptote is seen as an indicator of sampling sufficiency (Heck et al. 1975). While none of the curves actually reaches an asymptote, they appear to be approaching one and the rate of accumulation of species as successively more samples are added to the total is decreasing.

Species richness estimates

The procedure for fitting parametric models of relative abundance to a data set involves calculating the number of species expected in an abundance class and comparing it with the number of species actually observed. By summing the number of species expected (S^*), an estimate for species richness is obtained. The *Faraday* data fit both the log normal and truncated log normal models (Table 2), and the expected ethnospecies richness is 367 and 393 respectively. However, the log normal and truncated log normal models do not fit the other data sets and the expected ethnospecies richness for the *Black-owned* shops is unrealistically high (≈ 1016 ethnospecies for the log normal).

The log series model does not fit any of the data sets and there is an underestimation of the expected ethnospecies and species richness (Tables 2 and 3). The log

Table 2 Species richness estimates and other summary values for *ethnospecies* sold by shop traders and traders in the Faraday Street market

	Faraday street market	<i>Muti</i> shops		
		All shops	'Black-owned'	'Indian-owned'
No. of samples (<i>n</i>)	100	50	28	20
No. of individuals (<i>N</i>)	2402	4129	1769	2168
Observed <i>ethnospecies</i> richness	349	371	310	312
<i>Estimated ethnospecies richness</i>				
Chao 2	427.4±19.4	436.0±20.0	364.8±15.0	358.4±13.6
ICE	408.6	435.0	368.8	361.2
Jack 1	432.2±10.0	442.5±13.2	379.4±17.5	371.9±16.3
Jack 2	472.8	474.1	406.9	395.3
Bootstrap	388.0	404.4	343.4	340.3
MMMean	394.3	379.2	352.4	333.9
<i>Expected ethnospecies richness</i>				
Log normal	393.2*	698.5	1015.5	437.4
Truncated log normal	367.2*	386.5	320.1	319.9
Log series	(341.8)	(346.7)	(293.4)	(288.1)
No. of singletons	84	73	72	63
No. of doubletons	43	41	44	39

* $P \approx 0.21$

Each richness estimate represents the mean (and SD for some estimators) for 100 randomizations of the sample order (with the exception of the log normal and log series distributions). Number of samples (*n*) is the number of street market stalls or shops inventoried. Number of individuals (*N*) is the total number of individual plants recorded. Note: figures are for numbers of *ethnospecies* (i.e. common names) *not* species. Values in brackets estimated by the log series model are underestimates of the observed richness. Richness estimates for the subset of Black- and Indian-owned shops (shaded area) should ideally approximate the observed *ethnospecies* richness of 371 for the entire 'All shops' sample (shaded)

Table 3 Estimated *species* richness estimated based on multiplying the values in Table 2 by the ratio of observed *ethnospecies* to *species*

	Faraday street market	<i>Muti</i> shops		
		All shops	'Black-owned'	'Indian-owned'
Observed <i>ethnospecies</i> richness	349	371	310	312
Probable no. of corresponding species	≈475	≈508	≈425	≈427
Ratio of <i>ethnospecies</i> to species	1 to 1.36		1 to 1.37	
<i>Estimated species richness</i>				
Chao 2	581	587	500	491
ICE	556	596	505	495
Jack 1	588	606	520	510
Jack 2	643	650	557	395.3
Bootstrap	528	554	470	542
MMMean	536	520	483	457
Log normal	535	957	1391	599
Truncated log normal	499	530	320.1	438
Log series	(465)	(475)	(439)	(395)

For example, the 349 *ethnospecies* inventoried at the Faraday market corresponds with ≈475 species i.e. 1:1.36

series model, if it fits the data well, is reported to accurately predict the number of new species likely to be found in larger samples (Colwell and Coddington 1995). However, species richness will be underestimated if the data do not conform to the log series distribution (Magurran 2004). Furthermore, data that fit a log series have an implied low evenness because of the presumption that there is a large proportion of ‘rare’ species (i.e. singletons) and a small proportion of abundant species in the sample (Magurran 1988). Plant data frequencies recorded during the two surveys show the evenness to be very high (in the region of 0.91 to 0.99 for the Shannon J') (Williams et al. 2005). Therefore, these data won’t fit the log series distribution and the number of species not recorded during the sampling of the shops and stalls cannot be realistically predicted. An alternative is to use non-parametric methods to estimate species richness.

The best way to test the performance of a species richness estimator is to use data sets from sites where the actual species richness is known (Toti et al. 2000). Unfortunately, we cannot always use this approach successfully in ethnobotanical studies of large, regional ethnomedicinal markets such as Johannesburg because the plants sold in the markets originate from a wide geographical area of high species richness. Some plants sold in the Johannesburg markets are known to have been harvested in Zambia, Malawi and Mozambique. Furthermore, species entering commercial trade represent a ‘short-list’ of a far greater diversity of species used in rural areas (Cunningham 2001). The total number of species used for traditional medicine is thus much greater than a survey of a commercial market would ever capture.

There are, however, other ways to assess the usefulness of an estimator (Colwell and Coddington 1995; Toti et al. 2000). First, is to observe how rapidly the estimation curves approach an asymptote as sample size increases. Second, is to look for consensus among the estimators. And third, to compare the estimator curves to subjective visual extrapolations of the possible asymptotes of an observed species richness accumulation curve. A good estimator (1) should reach, or closely approach, a stable asymptote sooner (i.e. with fewer samples) than the observed species accumulation curve would, (2) yield an estimate that is not vastly different from the other estimators, and (3) should give estimates that are close to reasonable visual extrapolations of the asymptote of the observed species accumulation curve (Toti et al. 2000). However, not all curves will reach an asymptote, and if part of the reason for estimating species richness is to know the degree to which a site is ‘un-sampled’ and hence how many species remain to be discovered with further sampling, then another method for checking the efficiency of an estimator is to apply the ‘stopping rule’ i.e. the point beyond which further sampling is no longer necessary or too costly (Magurran 2004). Asymptotes are generally used to establish this point, but Magurran (2004) also proposes that a sample is subdivided into two parts and that the richness of each is estimated separately. If the estimates give answers that are consistent with the one obtained for the combined sample, then ample data have been collected (Magurran 2004). In the same way, the estimator that best predicts the species richness of the total sample from the subsamples can be adjudicated as the better estimator.

The Michaelis–Menton Mean (MMMean) estimator appears to be the only estimator that approaches an asymptote sooner than the expected accumulation curve, for all data sets (Fig. 3a–d). This result is probably to be expected given the asymptotic nature of the estimator (Magurran 2004). The remainder of the

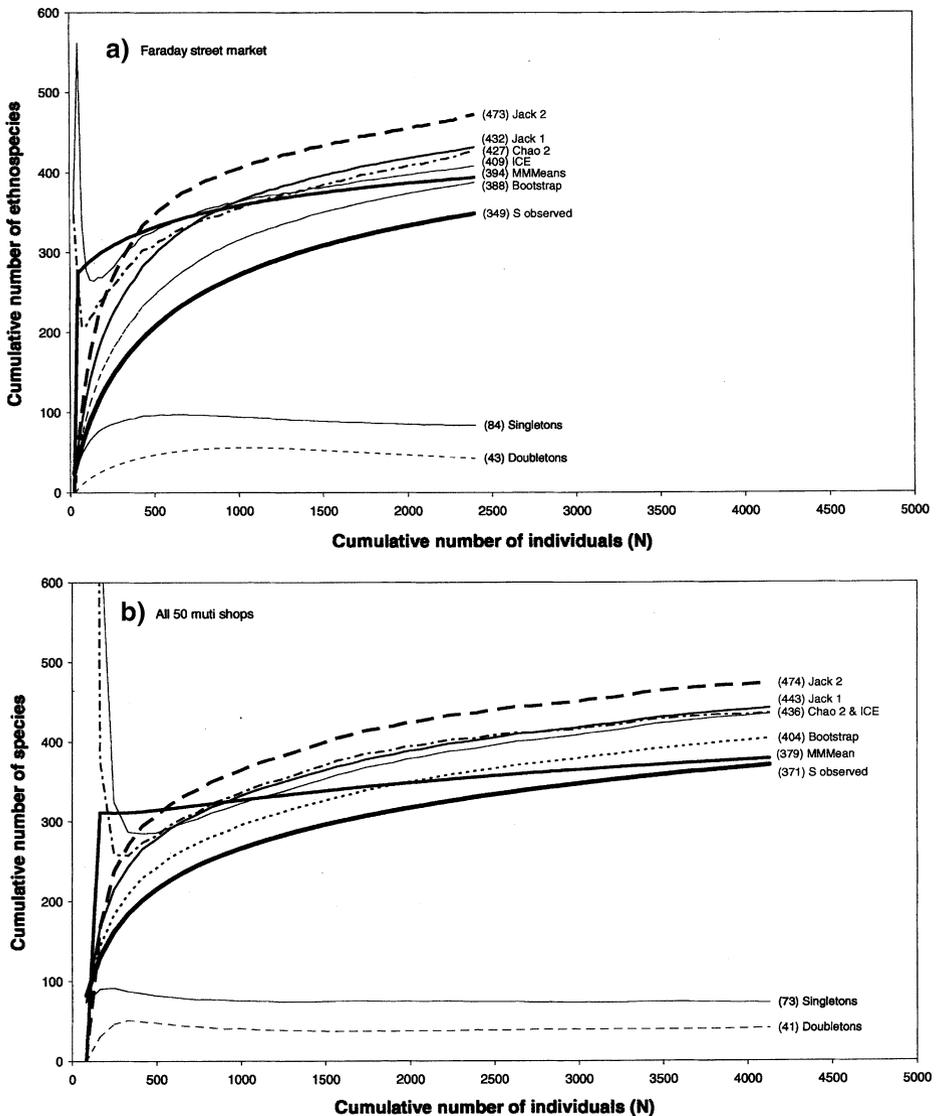


Fig. 3 The performance of the incidence-based species richness estimators compared with the expected species accumulation curve (S observed) for four data sets from the Faraday street market (**a**) and *multi* shops (**b–d**). The cumulative number of singletons and doubletons are also plotted. The curves above the species accumulation curve show the estimated species richness (final value in brackets) based on successively larger numbers of samples. The estimators used are Jack 1 & 2, Chao 2, ICE, MMMean and Bootstrap. The values were generated by *EstimateS* (Colwell 2005). For all curves, each point is the mean of 100 estimates based on 100 randomizations of the sample accumulation order. All graphs are scaled to the same axis values

estimators tend to rise in parallel with the accumulation curve and do not reach an asymptote. The first- and second-order Jackknife estimators climb more steeply than the other estimators, and Chao 2 and ICE over-estimate species richness at the beginning of the sample (because they are strongly affected by the high number of

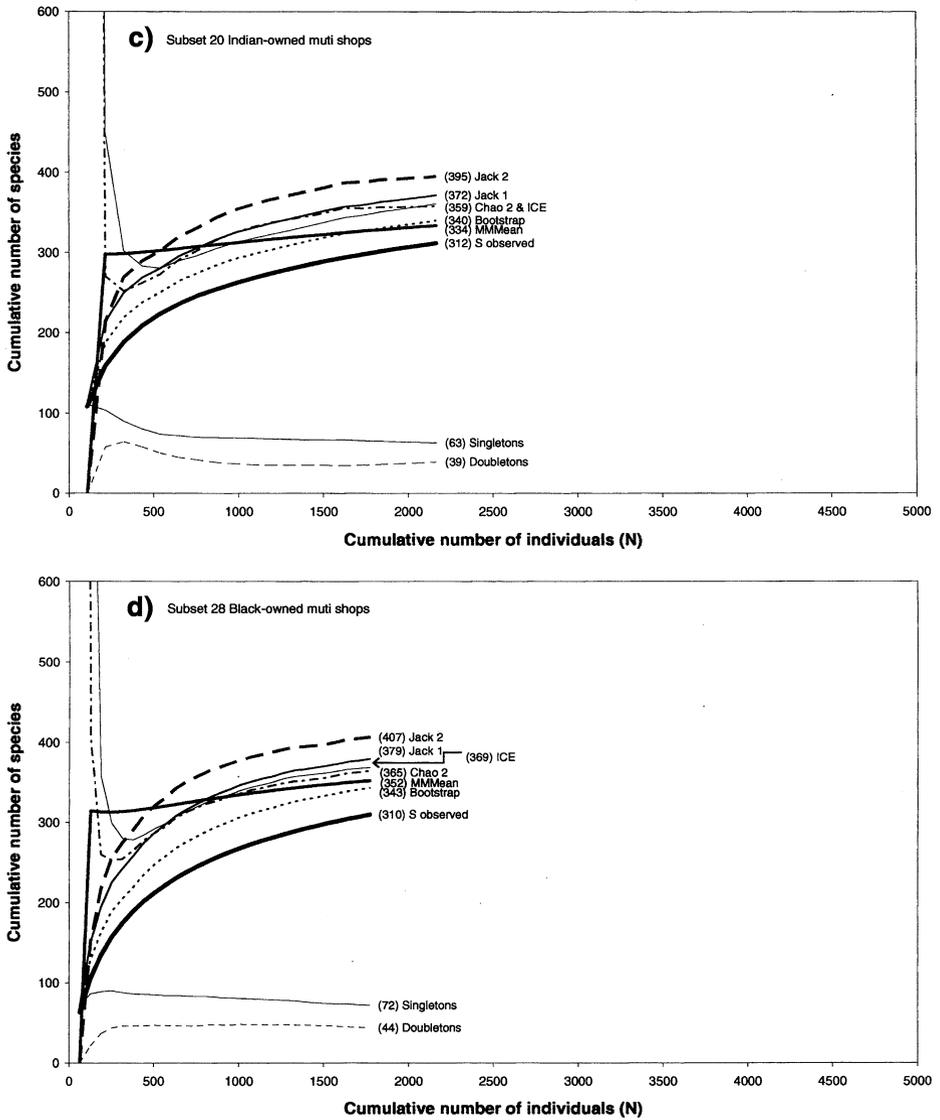


Fig. 3 continued

singletons and rare species present in the first few samples). Plots of singletons and doubletons rise quickly and then tend to level off and decrease very slightly as the sample size increases.

The difference between the highest and lowest ethnospecies estimate is 84.9 and 94.9 for *Faraday* and *All shops* respectively, and between 54.5 and 61.4 for the *Black-* and *Indian-owned* shops subset respectively (Table 2). Interestingly, the observed richnesses range from 74 to 79% of the maximum estimated value. The second- and first-order Jackknife estimators (Jack 2 and Jack 1) consistently generate the highest estimates, and either Bootstrap or MMEan the lowest estimates for the samples.

Despite the smaller number of species observed in the Faraday market, the species richness estimators predict that relatively more species are yet to be sampled in the Faraday market compared to the shops. This is due to the higher number of singletons recorded during the survey. Species richness estimators use the frequency of rare species to predict total sample species richness. The more singletons there are in a sample, the more species are predicted to be present in the sampling ‘universe’ that remain to be captured during a survey.

Overall, results indicate that based on the rate at which the estimators reach an asymptote, the MMMeans is the best estimator of species richness for the four ethnobotanical samples. However, the MMMeans estimates for species sold by the subset of *Indian-* and *Black-owned* shops (334 and 352 ethnospecies respectively) is less than the observed richness for the total number of ethnospecies inventoried in the 50 *muti* shops (i.e. 371 ethnospecies), of which the subsets are part (shaded area Table 2, Figure 3). Therefore, MMMeans was not able to correctly estimate the total richness of the total sample (*All shops*) based on the species present in either of the two data subsets. Instead, Jack 1 better predicts the species richness for the total sample (372 and 379 estimated ethnospecies for *Indian-* and *Black-owned* shops respectively, compared to 371 observed for *All shops*). Similarly, Jack 1 estimates for *Faraday* (Fig. 3a) and *All shops* (Fig. 3b) are close to those observed if the ethnospecies inventories of both samples are amalgamated to form a total species list for the region. When the two samples are combined (*WRand* in Table 4), the observed ethnospecies richness is 436 for 150 samples, which is similar to the 432 and 443 ethnospecies richness estimated by Jack 1 to be potentially present in either *Faraday* or *All shops* respectively. So, while MMMeans reaches an asymptote sooner and *appears* to be the best estimator for the data based on asymptote criteria, Jack 1 seems to provide the best lower-bound estimate of species richness for the individual data sets. If Jack 1 is the lower-bound estimator, then it is possible that Jack 2 estimates approach an upper limit for the number of species likely to be found in *commercial trade* in the region. The number of species actually used is far greater, but not all of them will find their way to the markets. While Chao 2 and ICE sometimes give estimates similar to that of Jack 1, the estimators are initially unstable and are therefore discounted.

Table 4 Ethnospecies richness and percentage complementarity between four data sets, including a study by Botha (2001) on medicinal plants traded on the western boundary of the Kruger National Park (*WBKP*)

	Faraday (<i>n</i> =100)	All Shops (<i>n</i> =50)	WRand Total (<i>n</i> =150)	WBKP (<i>n</i> =17)
Observed ethnospecies richness	349	371	436	185
<i>Complimentarity</i>				
All Shops	34.8% (284)			
WBKP	76.7% (101)	77.3% (103)	78.2% (111)	
<i>Combined richness for both sites</i>				
All Shops	436 (≈595)			
WBKP	453 (≈616)	473 (≈648)	510 (≈700)	

‘WRand Total’ is the combined data set of the Faraday and All Shop surveys. Values in brackets after the percent complementarity are the number of ethnospecies common on both data sets. Values in brackets after the combined ethnospecies richnesses are approximate numbers of corresponding species

A question of sampling sufficiency and the completeness of the inventories is raised because of the failure of most estimators (with the exception of MMMean and the *All shops* curve), and the species accumulation curves, to reach an asymptote. Curves that have an upward trend tend to indicate that an increase in sampling effort would yield more species, as is predicted by the estimators and the difference between the observed and estimated number of ethnospices. As regards the species accumulation curves, however, the rate of accumulation of new species is also necessary to make comparisons (Soberón and Llorente 1993), especially for certain ethnobotanical samples where the curves may never reach an asymptote because the commercial trade functions within a potentially very large regional floral diversity. Data for *Faraday* indicate that the rate of accumulation of new ethnospices is 0.9 per trader, whereas the rate for *All shops*, *Black-* and *Indian-*owned shops is 1.5, 2.7 and 3.5 respectively. These figures indicate the sampling effort was satisfactory, and that there would be no real benefit to interviewing more traders and inventorying their medicinal plant stock if, overall, <2 new ethnospices will be recorded per trader. Consequently, the sample size required for the species accumulation curves to reach an asymptote might be too large to be practical.

Heck et al. (1975) reported that in some situations it might be satisfactory to collect 50%–75% of the total number of species known to occur in a given area, as long as the most common species are recorded. While the total number of species in commercial medicinal plant trade is not precisely known, observed richness is $\geq 74\%$ of the maximum richness predicted by the estimators for all the samples and the most commonly traded species were captured by the survey. However, species represented as singletons/doubletons in an ethnobotanical sample derived from a commercial medicinal plant market are not necessarily ‘rare’ or ‘scarce’ in the wild. Instead, they often represent species that have little or no commercial value and/or have been speculatively harvested and sold to traders. As a result, the species richness estimators are, in principle, estimating total richness and the number of species that remain to be discovered from the preponderance of species with less commercial value.

Complimentarity and similarity

Values for the complimentarity and similarity indices show there to be a low level distinctness and high degree of similarity between the ethnospices sold in the *Faraday* market and *muti* shops (Tables 4 and 5). About 79% of the species sold in

Table 5 Jaccard and Sørensen’s coefficients of similarity for ethnospices sold, expressed as percentages

	Faraday (%)	All shops (%)	WRand total (%)	WBKP
<i>Classic Jaccard index</i>				
All shops	65.1			
WRand total	80.0	85.0		
WBKP	23.3	22.7	21.7	
<i>Classic Sørensen index (incidence-based)</i>				
All shops	78.9			
WRand total	88.9	91.9		
WBKP	37.8	37.0	35.7	

the market and shops are common to both samples (Sørensen index, Table 5), and the combined ethnospecies richness is 436 (≈ 595 species) (Table 4). The number of ethnospecies common to both samples is 284 (Table 4). Given that most shop traders purchase plants from the wholesale Faraday market and are in adjacent trading areas, the number of shared taxa and percentage similarity is expected to be high.

By contrast, comparing plants sold in the Witwatersrand and Mpumalanga markets (WBKP) (about 350 km apart) show a low degree of similarity and high degree of distinctness. Plants sold by the vendors in WBKP were 37% and 38% similar to the plants sold in *Faraday* and *All shops* respectively (Sørensen index, Table 5), and the combined ethnospecies richness was 453 (≈ 616 species) for WBKP and *Faraday*, and 473 (≈ 648 species) for WBKP and *All shops* (Table 4). When one combines the *Faraday* and *All shop* samples to produce an amalgamated data set (called 'WRand Total'), and compare it with WBKP, the Jaccard index of similarity is 36% and the shared number of ethnospecies is 510 (or ≈ 700 species). The number of ethnospecies common to both samples is relatively low (111 ethnospecies) and the two data sets are thus distinctly different. Given that $\leq 3\%$ of the plants sold in the Witwatersrand are harvested by gatherers in Mpumalanga, the dissimilarity of the two markets in terms of species richness is to be expected.

The resulting combined species richness for both sites (WRand and WBKP) (≈ 700 species) raises expectations for a high level of species richness commercially traded in and between different markets in South Africa. While no inventory data were available to compare the taxonomic similarities of species commercially traded in markets in KwaZulu-Natal (e.g. the Durban markets), the results would probably have shown the percent similarity to be greater than that of WBKP (i.e. $>40\%$) given that two-thirds of plants sold in the Johannesburg markets originate from KwaZulu-Natal. A factor that would additionally influence these results, however, would be the demand for medicinal plants harvested outside of South Africa (e.g. in Swaziland, Malawi and Mozambique). There are known to be noteworthy trade links between harvesters and markets for species found elsewhere in southern Africa (Williams 2005).

Conclusion

To enhance the value of ethnobotanical studies, the incorporation of suitable quantitative methods into data collection, processing and interpretation improves on the traditional compilation-style approach to the discipline (Höft et al. 1999) and, creates an opportunity for cogent arguments that advance scientific and practical knowledge. In the case of species accumulation functions, richness estimators and similarity measures, the methods broaden the interpretation of species inventories in several ways.

First, plotting species accumulation and rarefaction curves enables comparisons of species density per sample (i.e. the number of species per trader) and hence species richness at similar levels of sampling effort. When the axes are rescaled to individuals (N), the effect of density is removed and overall species richness between data sets can be compared. In addition, the 95% confidence intervals help assess whether the differences in species richness are significant. The shape of the curve, the rate of accumulation of new species and the degree to which curves approach a horizontal asymptote illustrate sampling sufficiency. It was clear from the results that *muti* shops stocked significantly more species than street traders did and that considerably

more sampling was required in the Faraday market before an equivalent level of species richness could be reached. Overall, the species richness was high. Even though the curves did not reach an obvious asymptote, the rate of accumulation of new species with increased sampling effort had levelled off enough for sampling effort to be considered satisfactory.

A well censused site is reportedly one where the species accumulation curve reaches a stable asymptote (Gotelli and Colwell 2001). However, given that the diversity of plants sold in traditional medicine markets is high (Williams et al. 2005) and the potential area from which plants can be harvested is extensive and continuously expanding (≈ 3 million km² including South Africa's immediate neighbours but excluding Namibia), there is some doubt as to whether the curve would *ever* reach a stable asymptote, especially if it hasn't done so after sampling 50 to 100 traders. The same doubt would apply to other ethnobotanical data sets, especially those derived from large, regional plant markets.

Second, is the investigative benefit of species richness estimation. A challenge with ethnobotanical species inventories and using richness estimators is that there are rarely survey scenarios where the actual species richness is known a priori. In the case of species traded in large regional markets such as Johannesburg, species are sold that were not only harvested in South Africa, but also in other southern African countries. Hence, it is difficult to test the performance of the richness estimator by comparing the results with data from sites where the actual species richness is known, because the total number of species that could be used cannot be realistically enumerated. That said, the estimators are still useful for predicting the total species richness of a site/assembly from a pooled set of samples.

The first-order Jackknife (Jack 1) correctly estimated the total ethnospecies richness of the *All shop* sample from the sub-samples of *Indian-* and *Black-owned* shops within 1–9 ethnospecies respectively. When the *Faraday* and *All shop* species inventories were combined ('WRand'), then the total number of ethnospecies was also similar to the Jack 1 estimate. Therefore, if Jack 1 is the best lower-bound estimator (because it predicted WRand richness), then the lower-bound estimate for the number of ethnospecies in commercial trade in the Witwatersrand ranges between 432 and 442 (equivalent to 588 and 606 species respectively). This indicates that the surveys captured at least 81% of the total number of species likely to be in commercial trade in the region. Alternatively, if Jack 2 is taken as the next best estimate to account for species not captured by either of the surveys, then total ethnospecies richness for the Witwatersrand traditional medicine trade is in the region of 473 ethnospecies or >643 species. The surveys would have thus accounted for $\approx 74\%$ of the total richness. This is more than adequate given Heck et al. (1975) recommended that the target should be 50%–75%. For the data sets investigated, the Jack 2 probably give an upper-bound approximation for the number of species most likely to be commercially traded in the region, including species that are speculatively harvested.

The least useful of the estimators are the species abundance models. Parametric models were originally developed to quantify abundance patterns that were evident in certain ecological communities and to test hypotheses about their underlying organization, including resource partitioning (Ludwig and Reynolds 1988; Magurran 1988). The generally poor fit of the data sets to the species distributions predicted by the models makes the rationale for using the models to estimate richness for ethnobotanical samples questionable. The relative abundances of ethnospecies in

ethnobotanical samples are not expected to have characteristics inherent in natural populations. Thus, the underlying assumptions that the models make a priori about the character of the data make the validity of fitting the models even more debatable. The log normal model is known to fit most 'large' data sets reasonably well (Hayek and Buzas 1997), and it is therefore probable that the good fit observed in the Faraday data set is due to the large number of samples. However, the definition of what constitutes an 'adequately large data set' is vague. The data sets investigated were large, but it was not possible to determine from the abundance models whether the richness estimates were upper- or lower-bound predictions. Therefore, collecting data adequate for fitting parametric models will not satisfactorily resolve the time and effort cost to benefit ratio for further sampling—which is one of the objectives when estimating species richness.

Third, is the usefulness of complementarity and similarity measures. They are the easiest of the measures to calculate and indicate the degree to which plant inventories compiled in different local and regional markets are distinct or similar. The percent similarity also infers the overall richness of species in commercial trade, especially between markets located in other parts of the country. If the WBKP sample has an observed ethnospecies richness of 185 and is only 36% similar to the combined WRand richness of 436, then potentially many more species exist along a 'trade gradient' between the two provinces. It would be similarly useful to compare the similarity of species sold in the markets of KwaZulu-Natal (e.g. Cunningham 1988) and the Eastern Cape (e.g. Dold and Cocks 2002), where the richness of species in trade is known to be high.

When using incidence-based species richness measures with ethnobotanical data, the least one should do is plot the sample-based rarefaction curves to compare the richness and density of the data sets at similar levels of sampling effort and assess the relative value of additional sampling. The benefits of estimating species richness, however, requires further consideration. Overall, the incidence-based measures described in this paper add value to the description of ethnobotanical species inventories and there is merit in applying these techniques to other case studies.

References

- Begossi A (1996) Use of ecological methods in ethnobotany. *Econ Bot* 50:280–289
- Bunge J, Fitzpatrick M (1993) Estimating the number of species: a review. *J Am Stat Assoc* 88:364–373
- Botha J (2001) Perceptions of availability and values of medicinal plants traded on the western boundary of the Kruger National Park, South Africa. MSc dissertation, University of the Witwatersrand, Johannesburg
- Chiarucci A, Enright NJ, Perry GLW, Miller BP, Lamont BB (2003) Performance of nonparametric species richness estimators in a high diversity plant community. *Divers Distrib* 9:283–295
- Colwell RK (2001) EstimateS: statistical estimation of species richness and shared species from samples. Version 6. User's guide and application published at: <http://www.viceroy.eeb.uconn.edu/estimates>
- Colwell RK (2005) EstimateS: statistical estimation of species richness and shared species from samples. Version 7.5. User's Guide and application published at: <http://www.purl.oclc.org/estimates>
- Colwell RK, Coddington JA (1995) Estimating terrestrial biodiversity through extrapolation. In: Hawksworth DL (ed), *Biodiversity, measurement and estimation*. Chapman and Hall, pp 101–118
- Colwell RK, Mao CX, Chang J (2004) Interpolating, extrapolating, and comparing incidence-based species accumulation curves. *Ecology* 85(10):2717–2727

- Cunningham AB (1988) An investigation of the herbal medicine trade in Natal-Kwazulu. Investigational Report No. 29, Institute of Natural Resources, University of Natal, Pietermaritzburg
- Cunningham AB (2001) Applied ethnobotany: people, wild plant use and conservation. People and Plants Conservation Manual, Earthscan, London
- Dold AT, Cocks ML (2002) The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African J Sci* 98(11–12):589–597
- Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett* 4:379–391
- Gotelli NJ, Entsminger GL (2001) EcoSim: null models software for ecology. Version 7.0. Acquired Intelligence Inc. and Kesity-Bear. <http://www.homepages.together.net/~gentsmin/ecosim.htm>
- Hanazaki N, Tamashiro JY, Leitão-Filho HF, Begossi A (2000) Diversity of plant use in two Caçara communities from the Atlantic Forest coast, Brazil. *Biodiv Conserv* 9:597–615
- Hayek LC, Buzas MA (1997) Surveying natural populations. Columbia University Press, New York
- Heck KL, van Belle G, Simberloff D (1975) Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. *Ecology* 56:1459–1461
- Höft M, Barik SK, Lykke AM (1999) Quantitative ethnobotany: applications of multivariate and statistical analysis in ethnobotany. People and Plants working paper 6. UNESCO, Paris
- Krebs CJ (1989) Ecological methodology. Harper and Row, New York
- Ludwig JA, Reynolds JF (1988) Statistical ecology—a primer on methods and computing. John Wiley and Sons, Toronto
- Magurran A (1988) Ecological diversity and its measurement. Princeton University Press, Princeton
- Magurran A (2004) Measuring biological diversity. Blackwell Publishing, Oxford
- Pielou EC (1975) Ecological diversity. Wiley InterScience, New York
- Soberón J, Llorente J (1993) The use of species accumulation functions for the prediction of species richness. *Conserv Biol* 7:480–488
- Sørensen L (2004) Composition and diversity of the spider fauna in the canopy of the montane forest in Tanzania. *Biodiv Conserv* 13:437–452
- Toti DS, Coyle FA, Miller JA (2000) A structured inventory of Appalachian grass bald and heath bald spider assemblages and a test of species richness estimator performance. *J Arachnol* 28:329–345
- Williams VL (2003) Hawkers of health: an investigation of the Faraday Street traditional medicine market in Johannesburg, Gauteng. Plant Ecology and Conservation Series No. 15, University of the Witwatersrand (Report to the Gauteng Directorate for Nature Conservation, DACEL), p 215
- Williams VL, Balkwill K, Witkowski ETF (1997) *Muthi* traders on the Witwatersrand, South Africa – an urban mosaic. *S Afr J Bot* 63(6):378–381
- Williams VL, Balkwill K, Witkowski ETF (2000) Unravelling the commercial market for medicinal plants and plant parts on the Witwatersrand, South Africa. *Econ Bot* 54(3):310–327
- Williams VL, Witkowski ETF, Balkwill K (2005) Application of diversity indices to appraise plant availability in traditional medicinal markets in Johannesburg, South Africa. *Biodiv Conserv* 14:2971–3001
- Williams VL (2005) Market networks for medicinal bark in southern Africa. In: Trees for health—forever: Implementing sustainable medicinal bark use in southern Africa. Regional Workshop, Johannesburg, South Africa, 1–3 November 2005. DFID-FRP Project R8305, UK