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Phylogenetic exploration of commonly used medicinal plants in South Africa

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Abstract

The rapid growth rate of human population, along with the public health crisis encountered in many regions, particularly in developing world, creates an urgent need for the discovery of alternative drugs. Because medicinal plants are not distributed randomly across lineages, it has been suggested that phylogeny along with traditional knowledge of plant uses can guide the identification of new medicinally useful plants. In this study, we combined different statistical approaches to test for phylogenetic signal in 33 categories of plant uses in South Africa. Depending on the null models considered, we found evidence for signal in up to 45% of plant use categories, indicating the need for multiple tests combination to maximize the chance of discovering new medicinal plants when applying a phylogenetic comparative approach. Furthermore, although there was no signal in the diversity of medicinal uses—that is, total number of medicinal uses recorded for each plant—our results indicate that taxa that are evolutionarily closely related have significantly more uses than those that are evolutionarily isolated. Our study therefore provides additional support to the body of the literature that advocates for the inclusion of phylogeny in bioscreening medicinal flora for the discovery of alternative medicines.

Keywords: Brownian motion model, *D* statistic, drug discovery, ethnomedicine, phylogenetic signal

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Introduction

The services provided by plants to humanity (here referred to as 'plant uses') are enormous. In traditional medicine in particular, the world known medicinal flora, estimated to ~10 000–53 000 species (Schippmann *et al.* 2002; McChesney *et al.* 2007), provides health care for ~80% of the global population in developing countries (Farnsworth *et al.* 1985). Also, natural plant products and their derivatives represent more than 50% of all drugs (Van Wyk *et al.* 1997) and could contribute ~70% to the small molecule antiinfective drugs (Newman & Cragg 2007). Furthermore, 25% of modern drugs are derived from plants traditionally used as medicine (Sahoo *et al.* 2010). In Africa, where access to modern medical facilities is limited (Elegami *et al.* 2002), the use of pharmaceutical drugs is an exception to the general trend (Koduru *et al.* 2007) as up to 90% of the African population depends directly on traditional medicine for treating illnesses (Staden 1999; Hostettmann *et al.* 2000; Fyhrquist 2007; WHO 2009). In South Africa, for example, ~80% of the population depend on traditional herbal medicine for their primary health care, with more than 25 000 practicing traditional healers (http://www.tac.org.za/Documents/ResearchPapers/Traditional_Medicine_briefing.pdf; accessed 24 March 2014). South Africa has a rich tropical and temperate flora, comprising ~24 000 species, which account for more than 10% of the globally known vascular plants (Germishuizen & Meyer 2003). Locally known medicinal plants represent approximately an eighth of this amazing plant diversity (Van Wyk & Gericke 2000), and several plants that can provide leads for new drug discoveries may therefore have been currently overlooked.

Given the immense societal benefits of traditional plant medicines, there has been a renewed interest in the search for new plants that might be medicinally useful in local ethnopharmacopoeias and perhaps for new drug discovery. Generally, pharmaceutical industries focus largely their efforts on methods such as synthetic chemistry and high-throughput screening. Such approaches are time-consuming and could perform better if screening can be focused more on lineages that are more probably to contain medicinally bioactive compound. For the purpose of facilitating focused bioscreening, one

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approach that is increasingly suggested in the literature is the investigation of the phylogenetic pattern of plant therapeutic uses across medicinal floras. Since the seminal study of Moerman (1991) which demonstrated taxonomic selectivity in the medicinal flora of North America, numerous studies have also shown that traditional uses of plants are not randomly distributed across plant groups. Instead, some groups of plants are overrepresented in local medicinal floras than others (e.g. Moerman *et al.* 1999; Leonti *et al.* 2003, 2013; Amiguet *et al.* 2006; Bennett & Husby 2008; Douwes *et al.* 2008; Saslis-Lagoudakis *et al.* 2011; Weckerle *et al.* 2011; de Medeiros *et al.* 2013). In addition, there is also mounting evidence of phylogenetic selectivity in traditional plant medicinal uses (Lukhoba *et al.* 2006; Forest *et al.* 2007; Rønsted *et al.* 2008, 2012; Saslis-Lagoudakis *et al.* 2011, 2012; Zhu *et al.* 2011). For example, a recent analysis of a comprehensive database of medicinal floras across different geographical regions (Nepal, New Zealand and South Africa) revealed that traditional plant uses are not dispersed randomly along the phylogeny of the regional floras; rather, they tend to cluster within some lineages across these disparate floras (Saslis-Lagoudakis *et al.* 2012). The identification of plant groups that stand out in ethnomedicine can lead to the underlying properties of these groups such as their organoleptic properties and ecological attributes (Leonti *et al.* 2003, 2013).

Beyond its potential to lead to lineages that are more prominent in local ethnopharmacopoeias, the phylogenetic perspective of ethnobotany has been proposed as a way of discovering new plants that have medicinal properties (Rønsted *et al.* 2008, 2012; Saslis-Lagoudakis *et al.* 2011, 2012).

Despite the importance of traditional medicine in the sustainability of health care for a large part of the world's population, its relevance for the discovery of new medicines has been a matter of controversy (Makhubu 1998; Fabricant & Farnsworth 2001; Firn 2003; Soejarto *et al.* 2005; Newman & Cragg 2012). Increasingly, however, there is advocacy for including traditional knowledge of herbal medicine in the search for new medicines (Balick 1990; Taniguchi & Kubo 1993; Cox & Balick 1994; Cox 2000; Fabricant & Farnsworth 2001; Lewis 2003; Newman & Cragg 2012). With the majority of the global flora not yet explored for its medicinal potential (Soejarto *et al.* 2005; Gurib-Fakim 2006)—particularly in tropical regions (Gurib-Fakim 2006)—prioritization of efforts is required to increase the success rates of biodiscovery schemes. The observation that medicinal properties in plants tend to be phylogenetically clustered has suggested that phylogenies can be used as a tool for prioritizing lineages to be investigated. Because species sharing similar evolutionary history (i.e. phylogenetically closely related species) generally share similar biochemical properties (Fairbrothers *et al.* 1975; Rønsted *et al.* 2008, 2012), mapping traditional uses and biochemical properties of plant species on phylogenetic trees could inform us on potentially new medicinal species and which lineages are particularly rich in these species. However, the relevance of phylogenetic approach in ethnomedicine has recently been questioned (Gertsch 2012), and the finding that biochemistry is not always phylogenetically clustered (e.g. Rønsted *et al.* 2012) indicates that this approach requires further exploration.

In this study, we investigate the phylogenetic patterns in commonly used medicinal plants in South Africa. We compile a list of commonly used medicinal plants and, using a series of methods describing the degree of phylogenetic clustering, we explore their distribution on the phylogeny of the Cape Floristic Region, a global biodiversity hotspot that harbours ~9000 plant species. Further, previous studies have indicated that phylogeny might also capture cultural importance of medicinal plants (Saslis-Lagoudakis *et al.* 2011; Leonti *et al.* 2013). As such, a strong phylogenetic pattern in cultural importance attached to species would be indicative of nonrandom selection of useful plants for traditional users, although knowledge of plant uses is dynamic (Saslis-Lagoudakis *et al.* 2014). As a measure of cultural importance, we used the diversity of medicinal uses (i.e. total number of uses recorded for each taxon). Although this approach is oversimplistic compared with more sophisticated approaches developed in ethnobotany (e.g. Trotter & Logan 1986; Prance *et al.* 1987; Bennett & Husby 2008; Tardío & Pardo-de-Santayana 2008; Thomas *et al.* 2009), these sophisticated metrics require additional information that is not available for this wide range analysis (e.g. numbers of species shown to participants to interviews; Thomas *et al.* 2009). Therefore, although a crude measure, we believe the diversity of uses conveys information about the cultural importance of a given taxon on the premise that more culturally important taxa might tend to have more uses (Prance *et al.* 1987).

In addition to testing phylogenetic signal in plant uses, we also explore the relationships between plant phylogenetic relatedness and plant cultural importance. The main aim of this study was to explore the contribution of phylogenetic methods in understanding the interactions between humans and floristic environments and in the biodiscovery of new plant medicines.

Material and methods

South African common medicinal plants and their uses

The medicinal flora of South Africa is estimated to ~650 species (Van Wyk & Gericke 2000), of which 132 species are identified as the 'most popular and widely used'

(Van Wyk *et al.* 1997). A recent study identified 16 additional plants as commonly used because of their wide use across the country (Mankga *et al.* 2013). In this study, we combined both lists to form a checklist of 148 species of commonly used medicinal plants distributed across 72 families *sensu* APG III (2009; Tables S1 and S2, Supporting information). Here, we referred to these species as the commonly used medicinal plants in South Africa. We conducted an extensive literature review of South African flora to document the common traditional uses of these plant species (Table S1, Supporting information). We then grouped all uses into 33 categories following (Mankga *et al.* 2013): diarrhoea, pneumonia, swellings, pains, wounds, coughs, fever, asthma, malaria, fertility, parasites, diabetes, arthritis, haemorrhoids, menorrhagia, gonorrhoea, laxative, leprosy, abortifacient, aphrodisiac, infections (skin and eye), sore throat, cancer, ulcers, ease childbirth, magic, food, cardiac problems, urinary complaints, nervous disorder, respiratory complaints, epilepsy and intestinal problems (Watt & Breyer-Brandwyijk 1962; Coates Palgrave 1983; Gelfand *et al.* 1985; Hutchings *et al.* 1996; Von Koenen 1996; Van Wyk & Van Wyk 1997; Van Wyk *et al.* 1997; Pooley 1998; Neuwinger 2000; Van Wyk & Gericke 2000; Schmidt *et al.* 2002; Dharani 2006; Lalitha *et al.* 2010). The proportions of uses recorded for each family are presented in Table S2 (Supporting information).

Phylogenetic tree of local flora

The phylogenetic tree used in our analyses was the genus-level phylogeny assembled for the Cape flora by Saslis-Lagoudakis *et al.* (2012), comprising 794 tips. The phylogeny was reconstructed using the plastid DNA rbcL sequences, which were analysed under the maximum-likelihood criterion based on the GTR + I + Γ model as implemented in RAXML 7.2.8 (Stamatakis *et al.* 2008).

Because this phylogeny is assembled at genus level, we allocated all 148 commonly used medicinal plant species to their respective genus. In a few cases where a genus included more than one commonly used medicinal species, this genus was only scored once. As a result, our data set comprises 142 genera.

Phylogenetic statistical analyses

All analyses were conducted in R (R Core Team 2013). Prior to analyses, we coded plant uses as binary traits: 1, when a species is used for the treatment of a particular ailment of the 33 categories and 0 when no medicinal use was recorded for a species among the 33 categories identified (Table S1, Supporting information). The following analyses were conducted.

Phylogenetic signal in medicinal uses

We tested for phylogenetic signal in plant uses using three alternative metrics: the net relatedness index (NRI) and the net taxon index (NTI) (Webb *et al.* 2002) implemented in the R package Picante (Kembel *et al.* 2010) and the *D* statistic (Fritz & Purvis 2010) implemented in the R package Caper (Orme *et al.* 2012) (see Data S1–S3, Supporting information for input data and R script). NRI describes the dispersion of traits (here plant uses) towards the root of the phylogeny, whilst NTI describes the patterns towards the tips. For both metrics, positive values indicate that closely related species have similar uses (phylogenetic clustering), whereas negative values indicate an even dispersion along the phylogeny. The significance of NRI and NTI was assessed comparing the observed patterns to the expectations after 10 000 randomizations (i.e. a null model of random shuffles along the entire phylogeny).

The *D* value for each medicinal use was calculated as follows:

$$D = \left[\sum d_{\text{obs}} - \text{mean}\left(\sum d_b\right)\right] \Big/ \left[\text{mean}\left(\sum d_r\right) - \text{mean}\left(\sum d_b\right)\right];$$

d_{obs} = observed, d = sum of absolute differences between the two ends of each branch in the phylogeny; d_r = expected d after 1000 random shuffles of traits along the phylogeny; and d_b = expected d under a Brownian motion (BM) model, that is, in a random walk with constant trait variance over time (Felsenstein 1985). $D = 1$ corresponds to a random distribution of uses across the tips; $D = 0$ indicates that plant uses are as clustered as one can expect under a BM model; $D < 0$ when medicinal uses are more clustered than expected by chance within certain plant lineages (strong phylogenetic signal), and $D > 1$ is indicative of a phylogenetic overdispersion (Fritz & Purvis 2010).

Phylogenetic signal in number of reported medicinal uses

In an earlier study (Lukhoba *et al.* 2006), it has been showed a correlation between phylogenetically delimited clades (within the genus *Plectranthus*) and number of plant uses such that highly diverse clade has more diverse medicinal uses. In addition, Forest *et al.* (2007) reconstructed a genus-level phylogenetic tree to show that an assemblage of plants with high phylogenetic diversity (here the sum of evolutionary history of individual genus) also shows higher number of medicinal uses (Forest *et al.* 2007), suggesting a link between

species evolutionary history and the diversity of services (here medicinal uses) that taxa provide to humanity, that is, 'ecosystem services' (see Faith *et al.* 2010). Based on these findings, we hypothesized that the diversity of plant medicinal uses (i.e. the number of uses recorded for each taxon) would correlate with phylogeny, that is, plant evolutionary history. We tested this hypothesis in two ways. First, we applied the *D* statistic to test for phylogenetic signal in diversity of plant use. For this purpose, we determined the 95th percentile of total uses recorded for all species in our data set and used this value as a threshold to convert the total number of uses per taxon into a binary trait, such that taxa whose total number of uses was below the threshold were arbitrarily categorized as 'less diversely used' and scored as 0 and those above the threshold as 'diversely used' and scored as 1. For sensitivity testing, the same categorization (less vs. diversely used) was carried out using the average diversity of plant uses in our data set (i.e. average number of uses for all 142 taxa across the data set) as threshold.

Second, we measured plant evolutionary history using Isaac *et al.*'s 2007 evolutionarily distinctiveness metric (ED). The metric ED describes the uniqueness, that is, the differences between species in terms of their evolutionary history and accounts for relationships between species towards the root of the phylogeny (Isaac *et al.* 2007). Again, the 95th percentile of ED values was used as a threshold to categorize taxa as highly vs. less distinct species (above and below the percentile, respectively). If there is a phylogenetic basis in diversity of uses, we would expect a strong relationship between ED categories (i.e. highly vs. less distinct species) and use categories (i.e. diversely used vs. less diversely used species). This correlation was assessed using Pearson's χ^2 test.

Results

Phylogenetic signal in medicinal uses

The most diverse families in medicinal uses in this study are Fabaceae (~13% of total uses recorded), Apocinaceae and Apiaceae (~5% each), whereas the least diverse families are Amaranthaceae, Caprifoliaceae and Rosaceae (~0.12% each; Table S2, Supporting information). In general, these medicinal uses are spread along the phylogeny (see Fig. 1), suggesting an overall phylogenetic overdispersion of plant uses. To test this, we conducted several tests of phylogenetic signal in plant uses. The results of these tests are presented in Table 1. Of the 33 medicinal uses analysed, the NRI metric identified only one significant clustered use (3% of total number of uses recorded), that is, treatment of 'intestinal problems'

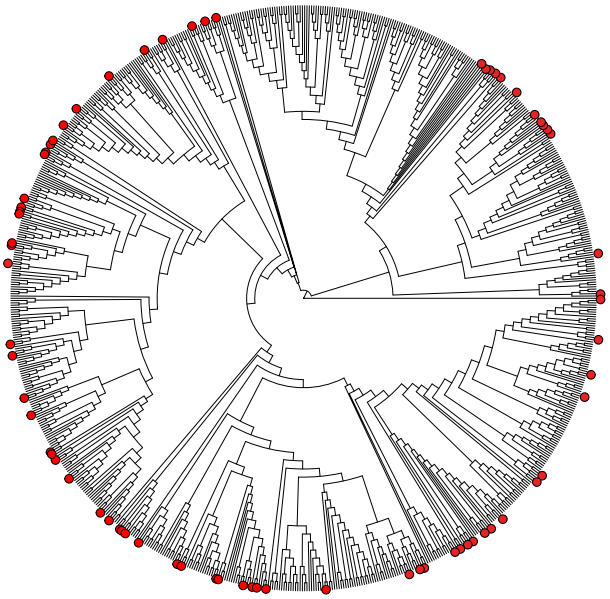

Fig. 1 Phylogeny of the Cape flora indicating the distribution of commonly used medicinal genera. This phylogeny is the genus-level phylogeny of Saslis-Lagoudakis *et al.* (2012). Medicinal genera as recorded in this study are indicated by the dots at the tips of the phylogeny.

(NRI = 2.89, $P = 0.001$). Similar clustering pattern was found for three plant uses (9% of all usages) when the phylogenetic structure was analysed using NTI metric: treatment of 'intestinal problems' (NTI = 1.83, $P = 0.034$), treatment of fever (NTI = 2.14, $P = 0.012$) and facilitation of childbirth (NTI = 1.68, $P = 0.036$).

Using the *D* statistic to compare the observed patterns to the random shuffle along the phylogeny, we found evidence for phylogenetic structure in only two uses (6% of all uses): treatment of fever ($D = 0.48$; $P = 0.024$) and strong signal in treatment of diabetes ($D = -1.018$; $P < 0.001$). However, when we compared the observed patterns to the expectations under a BM model, we found that 15 medicinal uses (45% of all uses) were as clustered on the phylogeny as expected under a BM model (Table 1). Overall, the vast majority of plant uses are not significantly clustered on the phylogeny, thus confirming the dispersion observed in Fig. 1.

Phylogenetic signal in diversity of medicinal uses

Testing the phylogenetic signal in diversity of uses (our simplistic measure of plant cultural importance), we found $D = 1.21$, which matches a random dispersion ($P = 0.83$) but differs significantly from 0, that is, BM expectation ($P = 0.00$), indicating the absence of phylogenetic structure. However, the diversity of uses was strongly associated with the evolutionary history of taxa,

Table 1 Results of phylogenetic signal tests

						P values	
Medicinal uses	NRI	*P* values	NTI	*P* values	*D* values	Random shuffle	Brownian motion
Diarrhoea	0.55	0.273	−1.26	0.903	0.81	0.23	0.017
Pneumonia	NA	NA	NA	NA	3.35	0.98	0.003
Swellings	0.59	0.28	−0.04	0.512	0.90	0.37	0.063
Pains	0.16	0.408	1.25	0.108	1.23	0.83	0.00
Wounds	−2.19	0.989	−1.12	0.877	0.98	0.45	0.002
Coughs	0.83	0.212	0.24	0.406	1.24	0.81	0.00
Fever	0.73	0.231	2.14	0.012	0.48	0.02	0.132
Asthma	−0.02	0.486	0.89	0.18	1.09	0.57	0.028
Malaria	−0.30	0.619	−0.35	0.653	1.40	0.91	0.00
Fertility	−0.41	0.658	−0.92	0.818	1.35	0.87	0.00
Parasites	−2.13	0.984	−1.44	0.927	0.85	0.28	0.023
Diabetic	−0.84	0.796	−0.63	0.736	−1.02	<0.001	0.854
Arthritis	−1.33	0.91	−1.95	0.981	1.50	0.82	0.029
Haemorrhoids	−0.83	0.78	−1.18	0.884	0.71	0.26	0.192
Menorrhagia	−0.91	0.831	−1.16	0.891	1.29	0.68	0.051
Gonorrhoea	0.76	0.213	0.07	0.458	0.93	0.42	0.11
Laxative	NA	NA	NA	NA	1.24	0.58	0.194
Leprosy	−0.004	0.513	−0.34	0.635	0.58	0.26	0.305
Abortifacient	1.44	0.078	0.67	0.24	0.49	0.13	0.288
Aphrodisiac	−0.31	0.617	0.04	0.449	1.06	0.52	0.072
Infections	−0.90	0.816	−0.89	0.815	1.21	0.81	0.00
Sore throat	−0.83	0.793	−1.06	0.855	0.39	0.11	0.342
Cancer	0.002	0.513	0.52	0.296	0.86	0.35	0.089
Ulcers	−0.026	0.507	0.52	0.29	0.73	0.14	0.041
Ease childbirth	0.72	0.249	1.68	0.036	1.17	0.70	0.007
Magic	−2.41	0.995	−1.05	0.852	1.63	0.86	0.005
Food	0.60	0.276	0.58	0.281	1.03	0.50	0.169
Cardiac problems	−0.82	0.796	1.02	0.158	0.91	0.40	0.057
Urinary complaints	−0.25	0.574	0.13	0.45	0.72	0.13	0.051
Nervous disorder	−0.007	0.494	−0.54	0.694	1.51	0.85	0.007
Respiratory complains	0.33	0.363	1.13	0.119	0.74	0.10	0.032
Epilepsy	0.87	0.193	0.20	0.416	0.81	0.25	0.046
Intestinal problems	2.89	0.001	1.83	0.034	0.92	0.33	0.004

NRI, net relatedness index; NTI, net taxon index; *D*, Fritz and Purvis' *D* statistic.

such that less distinct taxa, that is, closely related taxa tend to have more medicinal uses ($\chi^2 = 29.41$; d.f. = 1; $P < 0.001$; Figs 2 and S1).

Discussion

The phylogenetic comparative methods are increasingly applied to analyse ethnobotanical data (e.g. Lukhoba *et al.* 2006; Forest *et al.* 2007; Saslis-Lagoudakis *et al.* 2011, 2012) with the main aim of exploring the potential phylogenetic basis of traditional plant knowledge (Saslis-Lagoudakis *et al.* 2012). Our study contributes to this body of the literature by testing for phylogenetic signal in commonly used medicinal plants and the diversity of uses in South Africa. A recent study has provided a broader picture of phylogenetic ethnomedicine in South Africa's flora in comparison with other regional floras (Saslis-Lagoudakis *et al.* 2012). Similar to Saslis-Lagoudakis *et al.* (2012)'s study, we also tested for phylogenetic signal in plant medicinal uses in South Africa, but our study differs from theirs in two ways. First, we restrict our focus on only commonly used medicinal plants. As such, we are somewhat testing the relationships between phylogeny and plant cultural importance. Second, we also differ from theirs in our attempts to combine several phylogenetic statistical methods to explore signal. Overall, our findings show that the majority of commonly used medicinal plants were not significantly clustered on the phylogeny. The variation in the number of medicinal use categories that were clustered among methods is not surprising because different methods depict different evolutionary models (e.g. see Blomberg *et al.* 2003; Hardy *et al.* 2012), thus suggesting the need for multiple tests when applying phylogenetic comparative methods

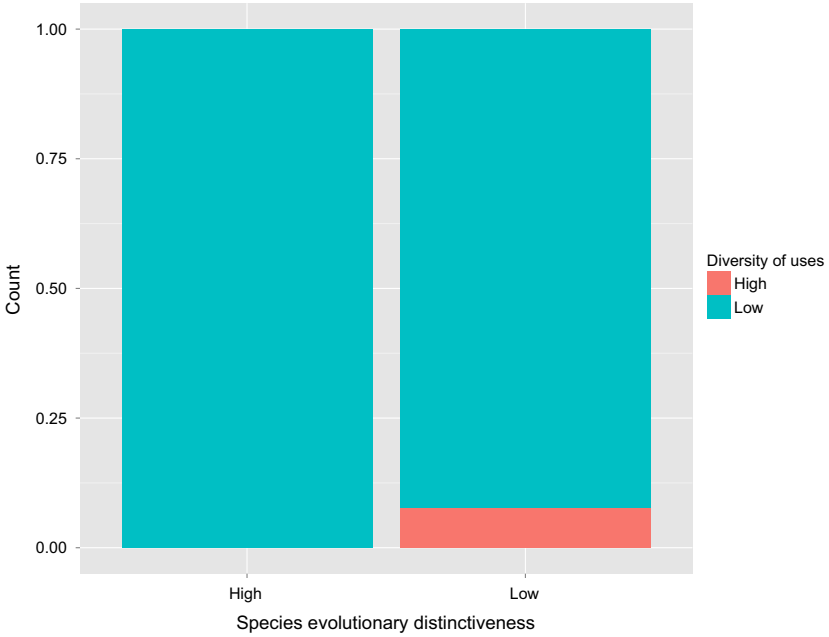

Fig. 2 Stacked histograms of the relationships between species evolutionary history and diversity of medicinal uses. Evolutionary history is measured here as species evolutionary distinctiveness (ED) following Isaac *et al.* (2007). As we did not use the complete phylogeny of Angiosperm species (which does not exist), the values of ED reported in this study for each taxa are valid only when considered within the context of the Cape flora. The categorization of high vs. low (for ED and diversity of plant uses) is delimited using the 95th percentile of values of each parameter (see also Fig. S1, Supporting information and Materials and Methods).

(Hardy *et al.* 2012) as the model that best describes trait evolution is generally unknown; at best, the model should be tested before applying a particular phylogenetic comparative method. The highest proportion of phylogenetically clumped medicinal uses (i.e. 45%) was found when comparing observed distribution of medicinal uses along the phylogeny with the expectation under a BM model. However, although the BM model has been assumed as null expectation in several studies (e.g. Blomberg *et al.* 2003), its universality has been discounted in many others (e.g. Mattila & Bokma 2008; Ackerly 2009; Yessoufou *et al.* 2012), further highlighting the need for multiple test comparisons.

Our results might seem contrary to that of Saslis-Lagoudakis *et al.* (2012) who found that ~54% of plant use categories in South Africa were significantly clumped on the phylogeny. However, our results are not directly comparable, for three reasons. First, we focused here on only commonly used plants (i.e. 142 of 650 medicinal taxa recorded in South Africa; Van Wyk & Gericke 2000), whilst Saslis-Lagoudakis *et al.*'s focus was broader. Second, our phylogenetic pool is also restricted to the Cape flora, as a phylogenetic tree of the whole of South Africa's flora (>20 000 species) was not available, whereas Saslis-Lagoudakis *et al.* (2012)'s pool was the combination of three disparate floras. Such restrictions could potentially blur the strength of phylogenetic signal in a given trait as the detection of signal may be influenced by the size of the pool considered (e.g. see Kraft *et al.* 2007). Third, our use categories are more partitioned than those in Saslis-Lagoudakis *et al.* (2012)—33 vs. 13 use categories.

We further explore the predictive power of phylogeny in ethnobotanical uses. We partitioned our data set of medicinal plants into two groups: species with high diversity of medicinal uses vs. species with low diversity, based on the number of medicinal uses recorded for each taxon. Using the *D* statistic, we did not find evidence for phylogenetic signal in diversity of uses, perhaps highlighting the influence of convergent evolution (or perhaps co-evolution) whereby secondary chemical compounds originate independently in taxa that are not phylogenetically closely related (Pichersky & Lewinsohn 2011). The vast majority of species included in this study have multiple uses. The absence of signal in number of uses could be a consequence of this multiple use of less related species.

However, when we evaluated the relationships between the evolutionary history of these two groups of species (species with high diversity of medicinal uses vs. species with low diversity), our results indicate that phylogenetically less distinct taxa (i.e. taxa with several closely relatives) tend to have more diverse medicinal uses than evolutionarily distinct taxa. This finding suggests that it is more probably to find taxa with more uses in species-rich clades, rather than in species-poor clades (see also Lukhoba *et al.* 2006 for medicinal use comparison between clade '*Coleus*' and the remaining clade of the genus *Plectranthus*) that might be represented with more long-branched taxa. One explanation for evolutionary distinct taxa having lower number of uses could be that older taxa might be less abundant in the flora, thus limiting knowledge on their medicinal properties. A recent analysis of the Cape flora in South Africa

indicated that phylogenetically younger species (phylogenetically less distinct taxa) are more threatened with risk of extinction (Davies *et al.* 2011), suggesting these species tend to be less common, perhaps as a result of human pressure.

Around the world, traditional medicine forms a significant source of primary health care. It is regarded as the most common and easily accessible source of medicine in most developing nations (Bello *et al.* 2011). The value of traditional medicine is even greater in African countries where the vast majority of the population relies almost exclusively on its services (Hostettmann *et al.* 2000; Newman *et al.* 2003). The amazing diversity of South African flora (~10% of the global angiosperm diversity) could be a more important source of new medicines if continued commitment is devoted to the search for new medicinal plants (Van Wyk 2011). There are several evidences that traditional knowledge of medicinal plants is useful in the search for new medicines (Balick 1990; Taniguchi & Kubo 1993; Cox 2000; Lewis 2003; Harvey 2008; Sehgal *et al.* 2012). Recently, the use of phylogenetic approach has been proposed as a potential guiding tool to facilitate and accelerate this search (Rønsted *et al.* 2008, 2012; Saslis-Lagoudakis *et al.* 2011, 2012). This is based on the principle that, because underlying chemistry is evolutionarily conserved (Agrawal *et al.* 2009; Rønsted *et al.* 2012), directing searches for new medicines within lineages identified as prominent in traditional medicine, could enhance outcomes. However, although phylogeny is assumed to capture ecosystem services ('evosystem services'; Faith *et al.* 2010) including medicinal uses (Forest *et al.* 2007), several authors have indicated that caution must be exercised over the specific trait and scale under consideration (e.g. Rønsted *et al.* 2012; Winter *et al.* 2013; Kelly *et al.* 2014).

Our study aimed to investigate the generality of phylogenetic patterns in traditional herbal medicine, by exploring evolutionary relationships of commonly used medicinal plants of South Africa. Whatever the methods applied, we consistently found evidence for phylogenetic clustering in some use categories, but never for the majority. Our findings indicate that, at least for commonly used medicinal plants, there is no evidence of phylogenetic signal in the majority of plant uses as shown in different studies that apply different methods (Forest *et al.* 2007; Saslis-Lagoudakis *et al.* 2012; and this study). Hence, the present work suggests that further exploration of ethnobotanical uses in a phylogenetic context is needed before the generality of the phylogenetic pattern in ethnobotany is globally accepted. Finally, we found that taxa with more uses are randomly distributed on the phylogeny, but are generally not evolutionarily distinct. This finding receives support from the ethnobotanical literature, which shows that species-rich, widespread taxa are more probably to be included in local ethnopharmacopoeias (Stepp & Moerman 2001; Leonti *et al.* 2013). These observations demonstrate how phylogenetic tools can reveal underlying patterns in plant selection by human communities. However, we believe these explorations highlight the need for reliable ethnobotanical data and therefore advocate for further financial support for ethnobotanical studies. This is particularly important as there is a widespread tread of erosion of traditional knowledge on plant uses worldwide (Voeks & Leony 2004; Srithi *et al.* 2009).

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References

Ackerly DD (2009) Conservatism and diversification of plant functional traits: evolutionary rates versus phylogenetic signal. *Proceedings of the National Academy of Sciences, USA*, **106**(Suppl. 2), 19699–19706.

Agrawal AA, Salminen J-P, Fishbein M (2009) Phylogenetic trends in phenolic metabolism of milkweeds (*Asclepias*): evidence for escalation. *Evolution*, **63**, 663–673.

Amiguet VT, Arnason JT, Maquin P *et al.* (2006) A regression analysis of q'eqchi' Maya medicinal plants from southern Belize. *Economic Botany*, **60**, 24–38.

APG III (2009) An update of APG classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society*, **161**, 105–121.

Balick MJ (1990) Ethnobotany and the identification of therapeutic agents from the rainforest. In: *Bioactive Compounds from Plants* (eds Chadwick DJ, Marsh J), pp. 22–39. John Wiley & Sons, Chichester.

Bello A, Aliero AA, Saidu Y, Muhammad S (2011) Phytochemical screening, polyphenolic content and alpha-glucosidase inhibitory potential of *Leptadenia hastata* (Pers.) Decne. *Nigerian Journal of Basic and Applied Science*, **19**, 181–186.

Bennett BC, Husby CE (2008) Patterns of medicinal plant use: an examination of the Ecuadorian Shuar medicinal flora using contingency table and binomial analyses. *Journal of Ethnopharmacology*, **116**, 422–430.

Blomberg SP, Garland T, Ives AR (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, **57**, 717–745.

Coates Palgrave K (1983) *Trees of Southern Africa*, 2nd edn. Struik Publishers, Cape Town.

Cox PA (2000) Will tribal knowledge survive the millennium? *Science*, **287**, 44–45.

Cox PA, Balick MJ (1994) The ethnobotanical approach to drug discovery. *Scientific American*, **270**, 82–87.

Davies TJ, Smith GF, Bellstedt DU *et al.* (2011) Extinction risk and diversification are linked in a plant biodiversity hotspot. *PLoS Biology*, **9**, e1000620.

Dharani N (2006) *Field Guide to Acacias of East Africa*. Struik Publishers, Cape Town, South Africa.

Douwes E, Crouch NR, Edwards TJ, Mulholland DA (2008) Regression analyses of southern African ethnomedicinal plants: informing the tar-

geted selection of bioprospecting and pharmacological screening subjects. *Journal of Ethnopharmacology*, **119**, 356–364.
Elegami AA, El-Nima EI, El-Tohami MS, Muddathir AK (2002) Antimicrobial activity of some species of the family Combretaceae. *Phytomedicine*, **16**, 555–561.
Fabricant DS, Farnsworth NR (2001) The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, **109**, 69–75.
Fairbrothers DE, Mabry TJ, Scogin RL, Turner BL (1975) Bases of angiosperm phylogeny – chemotaxonomy. *Annals of the Missouri Botanical Garden*, **62**, 765–800.
Faith DP, Magallón S, Hendry AP, Conti E, Yahara T, Donoghue MJ (2010) Evosystem services: an evolutionary perspective on the links between biodiversity and human well-being. *Current Opinion in Environmental Sustainability*, **2**, 66–74.
Farnsworth NR, Akerele O, Bingel AS (1985) Medicinal plants in therapy. *Bulletin of the World Health Organization*, **63**, 965–981.
Felsenstein J (1985) Phylogenies and the comparative method. *The American Naturalist*, **125**, 1–15.
Firn RD (2003) Bioprospecting – why is it so unrewarding? *Biodiversity Conservation*, **12**, 207–216.
Forest F, Grenyer R, Rouget M *et al.* (2007) Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature*, **445**, 757–760.
Fritz SA, Purvis A (2010) Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conservation Biology*, **24**, 1042–1051.
Fyhrquist A (2007) *Traditional Medicinal Uses and Biological Activities of Some Plant Extracts of Africa Combretum Loefl., Terminalia L and Pteleopsis Engl. species (Combretaceae)*. Yliopistopaino, Helsinki.
Gelfand M, Mavi S, Drummond RB, Ndemera B (1985) *The Traditional Medical Practitioner in Zimbabwe*. Mambo Press, Gweru, Zimbabwe.
Germishuizen G, Meyer NL (2003) *Plants of Southern Africa: Annotated Checklist Strelitzia 14*. National Botanical Institute, Pretoria.
Gertsch J (2012) Cross-cultural comparisons of medicinal floras—What are the implications for bioprospecting? *Journal of Ethnopharmacology*, **139**, 685–687.
Gurib-Fakim A (2006) Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, **27**, 1–93.
Hardy C, Fara E, Laffont R *et al.* (2012) Deep-time phylogenetic clustering of extinctions in an evolutionarily dynamic clade (Early Jurassic Ammonites). *PLoS One*, **7**, e37977.
Harvey AL (2008) Natural products in drug discovery. *Drug Discovery Today*, **13**, 894–901.
Hostettmann K, Marston A, Ndjoko K, Wolfender JL (2000) The potential of African plants as a source of drugs. *Current Organic Chemistry*, **4**, 973–1010.
Hutchings A, Scott AH, Lewis G, Cunnignham AB (1996) *Zulu Medicinal Plants*. University of Natal Press, Scottsville, South Africa.
Isaac NJB, Turvey ST, Collen B, Waterman C, Baillie JEM (2007) Mammals on the EDGE: conservation priorities based on threat and phylogeny. *PLoS One*, **2**, e296.
Kelly S, Grenyer R, Scotland RW (2014) Phylogenetic trees do not reliably predict feature diversity. *Diversity and Distributions*, **20**, 600–612.
Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H (2010) Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, **26**, 1463–1464.
Koduru S, Grierson DS, Afolayan AJ (2007) Ethnobotanical information of medicinal plants used for the treatment of cancer in the Eastern Cape province, South Africa. *Current Science*, **92**, 906–908.
Kraft NJ, Cornwell WK, Webb CO, Ackerly DD (2007) Trait evolution, community assembly, and the phylogenetic structure of ecological communities. *The American Naturalist*, **170**, 271–283.
Lalitha S, Rajeshwaran K, Kumar PS, Deepa K, Gowthami K (2010) In vivo of antibacterial activity of *Senegalia mellifera* (BENTH) (Leguminodae) on Human Pathogenic Bacteria. *Global Journal of Pharmacology*, **4**, 148–150.
Leonti M, Ramirez RF, Sticher O, Heinrich M (2003) Medicinal flora of the Popoluca, Mexico: a botanical systematical perspective. *Economic Botany*, **57**, 218–230.
Leonti M, Cabras S, Castellanos ME, Challenger A, Gertsch J, Casu L (2013) Bioprospecting: evolutionary implications from a post-olmec pharmacopoeia and the relevance of widespread taxa. *Journal of Ethnopharmacology*, **147**, 92–107.
Lewis W (2003) Pharmaceutical discoveries based on ethnomedicinal plants: 1985–2000 and beyond. *Economic Botany*, **57**, 126–134.
Lukhoba CW, Simmonds MSJ, Paton AJ (2006) *Plectranthus*: a review of ethnobotanical uses. *Journal of Ethnopharmacology*, **103**, 1–24.
Makhubu L (1998) Bioprospecting in an African context. *Science*, **282**, 41–42.
Mankga LT, Yessoufou K, Moteetee AM, Daru BH, van der Bank M (2013) Efficacy of the core DNA barcodes in identifying processed and poorly conserved plant materials commonly used in South African traditional medicine. *Zookeys*, **365**, 215–233.
Mattila TM, Bokma F (2008) Extant mammal body masses suggest punctuated equilibrium. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2195–2199.
McChesney JD, Venkataraman SK, Henri JT (2007) Plant natural products: back to the future or into extinction? *Phytochemistry*, **68**, 2015–2022.
de Medeiros PM, Ladio AH, Santos AMM, de Albuquerque UP (2013) Does the selection of medicinal plants by Brazilian local populations suffer taxonomic influence? *Journal of Ethnopharmacology*, **146**, 842–852.
Moerman DE (1991) The medicinal flora of native North-America – an analysis. *Journal of Ethnopharmacology*, **31**, 1–42.
Moerman DE, Pemberton RW, Kiefer D, Berlin B (1999) A comparative analysis of five medicinal floras. *Journal of Ethnobiology*, **19**, 49–67.
Neuwinger HD (2000) *African Traditional Medicine: A Dictionary of Plants Use and Applications*. Medplasm Scientific Publishers, St Leon-Rot, Germany.
Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 Years. *Journal of Natural Products*, **70**, 461–477.
Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products*, **75**, 311–335.
Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period 1981–2002. *Journal of Natural Products*, **66**, 1022–1037.
Orme D, Freckleton R, Thomas G *et al.* (2012) *caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5*. http://CRAN.R-project.org/package=caper.
Pichersky E, Lewinsohn E (2011) Convergent evolution in plant specialized metabolism. *Annual Review of Plant Biology*, **62**, 549–566.
Pooley E (1998) *A Field Guide to Wild Flowers KwaZulu-Natal and the Eastern Region*. Natal Flora Publications Trust, Durban, South Africa.
Prance GT, Balee W, Boom BM, Carneiro RL (1987) Quantitative ethnobotany and the case for conservation in Ammonia. *Conservation Biology*, **1**, 296–310.
R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
Rønsted N, Savolainen V, Mølgaard P, Jager AK (2008) Phylogenetic selection of *Narcissus* species for drug discovery. *Biochemical Systematics and Ecology*, **36**, 417–422.
Rønsted N, Symonds MRE, Birkholm T *et al.* (2012) Can phylogeny predict chemical diversity and potential medicinal activity of plants? A case study of amaryllidaceae. *BMC Evolutionary Biology*, **12**, 182–193.
Sahoo N, Manchikanti P, Dey S (2010) Herbal drugs: standards and regulation. *Fitoterapia*, **81**, 462–471.
Saslis-Lagoudakis CH, Klitgaard BB, Forest F *et al.* (2011) The use of phylogeny to interpret cross-cultural patterns in plant use and guide medicinal plant discovery: an example from *Pterocarpus* (Leguminosae). *PLoS One*, **6**, e22275.

Saslis-Lagoudakis CH, Savolainen V, Williamson EM *et al.* (2012) Phylogenies reveal predictive power of traditional medicine in bioprospecting. *Proceedings of the National Academy of Sciences, USA*, **109**, 15835–15840.

Saslis-Lagoudakis CH, Hawkins JA, Greenhill SJ *et al.* (2014) The evolution of traditional knowledge: environment shapes medicinal plant use in Nepal. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20132768.

Schippmann U, Leaman DJ, Cunningham AB (2002) . *Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Satellite Event on the Occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food and Agriculture Rome, 12–13 October* (ed. Inter-Departmental Working Group on Biological Diversity for Food and Agriculture). Food and Agriculture Organization, Rome.

Schmidt E, Lotter M, McCleland W (2002) *Trees and Shrubs of Mpumalanga and Kruger National Park*. Jacana Media, Johannesburg, South Africa.

Sehgal N, Gupta A, Valli RK *et al.* (2012) *Withania somnifera* reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. *Proceedings of the National Academy of Sciences, USA*, **109**, 3510–3515.

Soejarto DD, Fong HH, Tan GT *et al.* (2005) Ethnobotany/ethnopharmacology and mass bioprospecting: issues on intellectual property and benefit-sharing. *Journal of Ethnopharmacology*, **100**, 15–22.

Srithi K, Balslev H, Wangpakapattanawong P, Srisanga P, Trisonthi C (2009) Medicinal plant knowledge and its erosion among the Mien (Yao) in northern Thailand. *Journal of Ethnopharmacology*, **123**, 335–342.

Staden JV (1999) Medicinal plants in southern Africa: utilization, sustainability, conservation – can we change mindsets? *Outlook on Agriculture*, **28**, 75–76.

Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, **57**, 758–771.

Stepp JR, Moerman DE (2001) The importance of weeds in ethnopharmacology. *Journal of Ethnopharmacology*, **75**, 19–23.

Taniguchi M, Kubo I (1993) Ethnobotanical drug discovery based on medicine men's trials in the African savanna: screening of east African plants for antimicrobial activity II. *Journal of Natural Products*, **56**, 1539–1546.

Tardío J, Pardo-de-Santayana M (2008) Cultural importance indices: a comparative analysis based on the useful wild plants of Southern Cantabria (Northern Spain). *Economic Botany*, **62**, 24–39.

Thomas E, Vandebroek I, Sanca S, Van Damme P (2009) Cultural significance of medicinal plant families and species among Quechua farmers in Apillapampa, Bolivia. *Journal of Ethnopharmacology*, **122**, 60–67.

Trotter RT, Logan MH (1986) Informant consensus: a new approach for identifying potentially effective medicinal plants. In: *Plants in Indigenous Medicine and Diet Biobehavioral Approaches* (ed. Etkin NL), pp. 91–112. Redgrave Publishing Co, Bedford Hills, NY.

Van Wyk B-E (2011) The potential of South African plants in the development of new medicinal products. *South African Journal of Botany*, **77**, 812–829.

Van Wyk B-E, Gericke N (2000) *People's Plants: A Guide to Useful Plants of Southern Africa*. Briza Publications, Pretoria.

Van Wyk B-E, Van Wyk P (1997) *Field Guide to Trees of South Africa*. Struik Publishers, Cape Town.

Van Wyk B-E, Van Oudtshoorn B, Gericke N (1997) *Medicinal plants of South Africa*, 1st edn. Briza Publications, Pretoria.

Voeks R, Leony A (2004) Forgetting the forest: assessing medicinal plant erosion in Eastern Brazil. *Economic Botany*, **58**, S294–S306.

Von Koenen E (1996) *Medicinal Poisonous and Edible Plants in Namibia*. Klaus Hess Publishers, Namibia.

Watt JM, Breyer-Brandwyijk MG (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd edn. Livingstone, London.

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ (2002) Phylogenies and community ecology. *Annual Review of Ecology, Evolution, and Systematics*, **33**, 475–505.

Weckerle CS, Cabras S, Castellanos ME, Leonti M (2011) Quantitative methods in ethnobotany and ethnopharmacology: considering the overall flora—Hypothesis testing for over- and underused plant families with the Bayesian approach. *Journal of Ethnopharmacology*, **137**, 837–843.

WHO (2009) *WHO Launches the First Strategy on Traditional and Alternative Medicine*. http://www.who.int/mediacentre/news/releases/release38/en/.

Winter M, Devictor V, Schweiger O (2013) Phylogenetic diversity and nature conservation: where are we? *Trends in Ecology & Evolution*, **28**, 199–204.

Yessoufou K, Abalaka J, van der Bank M, Daru BH (2012) Fig-frugivore interactions follow a constrained Brownian motion model of evolution in an Important Bird Area, West Africa. *Israel Journal of Ecology and Evolution*, **58**, 39–51.

Zhu F, Qin C, Tao L *et al.* (2011) Clustered patterns of species origins of nature-derived drugs and clues for future bioprospecting. *Proceedings of the National Academy of Sciences, USA*, **108**, 12943–12948.

K.Y. designed and performed the research; K.Y. and B.H.D. contributed new reagents or analytical tools; K.Y. analysed the data; K.Y. wrote the article along with significant contributions from B.H.D. and A.M.M.

Data accessibility

Details of DNA data and phylogeny used in this study can be found under TreeBase ID# 16147. In addition, all the medicinal uses are presented in Table S1.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Stacked histograms of the relationships between species evolutionary history and diversity of medicinal uses.

Table S1 Plant species and their medicinal uses recorded in South Africa.

Table S2 Proportion of total uses recorded for all families of commonly used medicinal plants in South Africa.

Data S1 Data matrix of medicinal uses.

Data S2 Categorization of ED and diversity of uses as high and low (see text for details).

Data S3 R codes used for data analysis.