The Future of Drug Discovery: Are collections needed?

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Abstract

Collections serve as repositories documenting the distribution of plants across time and space. At the same time, collections, both living and preserved, are an immense source of 'big data' for a wide range of research applications from the core discipline of taxonomy to testing evolutionary relationships in the 'genomics era', drivers of biodiversity, and the highly topical impact of environmental change. In this paper, we argue that collections are also essential for medicinal plant research and have the potential to significantly impact modern drug lead discovery. Collections are, at the very least, needed to allow authoritative identification and documentation of medicinal or any other plant material. Additionally, collections provide a powerful framework for understanding variation of natural products at all scales from ecological or chemical types within species, to chemical diversity within lineages and across the entire plant domain. Examples from recent studies are that DNA barcoding can be used for authentication of *Equisetum arvense* products, and collections can provide easily accessible high quality samples for creating barcoding reference libraries. Medicinal uses of *Aloe* has been correlated with the phylogeny and succulence of the leaves, and the origin of now globally popular *Aloe vera* could be traced to the Arabian Peninsula, suggesting a connection with ancient trade routes. Using collections provide easy access to biodiversity for improving selection and focusing drug lead discovery efforts, avoid destructive collection of rare and threatened species, and provide added value to collections. However, new collections are needed in medicinal plant research, requiring additional efforts and permits to ensure compliance with international conventions and creating added synergy of North-South collaborations.

Key Words: authentication, herbaria, medicinal plants, phylogenetic selection, intra-specific variation

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In the face of a long list of unmet medical needs one of the grand challenges for society today is the identification of new leads by pharmaceutical companies, which can improve and restore health and welfare. Historically, plants have been very important sources of medicine, but most companies now focus entirely on synthetic libraries to identify new leads. At the same time, the number of new drugs brought to the market is steadily declining, notwithstanding the progress in design and discovery technologies during the recent decades (Scannell *et al.* 2012). Nevertheless, among twenty new chemical entities launched worldwide to the pharmaceutical market in 2010, ten (50%) are natural products (Newman & Cragg 2012). This trend is evident also in the longer perspective, as shown by Newman, Cragg and co-workers in a series of papers (Cragg & Newman 2009, 2013; Newman & Cragg 2012) and demonstrates that modern natural product based drug-discovery programs are highly productive and competitive when compared to other drug discovery technologies.

Plants provide healthcare worldwide. Twenty-five percent of modern medicines originate from plants originally used in traditional medicine, and as much as 50% of all drugs can be traced back to natural resources (Cragg & Newman 2009). A recent example is ingenol mebutate (from *Euphorbia peplus* L.) registered as a drug for treatment of actinic keratosis by LEO Pharma A/S (Berman 2012). Evolutionary processes have led plants and other organisms to develop a diversity of chemical defenses selected for their biological activity (Ehrlich & Raven 1964). Furthermore, plant metabolites are made by living organisms and consequently have an affinity for functional proteins from the outset. It is also shown that chemical diversity found in natural sources greatly outperforms that of the synthetic libraries, thus increasing the likelihood of new drug lead discovery from nature (Larsson *et al.* 2007). However, there is a range of challenges associated with drug discovery from biodiversity resources. Identification and isolation of bioactive compounds are both time-consuming and costly and the number of known as well as yet unclassified biological species, including plants, is enormous.

Whereas methods for isolation and identification of natural products have greatly advanced in recent decades, methods for the selection of target plants have hardly developed. Large-scale drug discovery programs such as the one run by the National Cancer Institute (USA) during the period 1960–1982 basically screened material from all plants available to them at random. The important new drug paclitaxel (taxol, from the Pacific Yew tree, *Taxus brevifolia* Nutt.) used in anticancer treatment, emerged from such programs (Cragg & Newman 2009; Newman & Cragg 2012). Although this is a success story of random screens, this approach can be extremely inefficient as there is no indication on the potential bioactivity of the selected plant material. To increase efficiency, academic efforts have largely focused on taking advantage of knowledge from traditional medicine to choose plants for further investigation (Bohlin *et al.* 2012). By using a systematic and integrative approach to explore the great inventiveness and diversity of na-

ture, we may potentially provide a more efficient way of finding new and improved leads for drug development from biodiversity resources (Rønsted *et al.* 2008, 2012; Bohlin *et al.* 2012). As biodiversity is decimated by changing land use, climate change and over-exploitation, identifying the species most likely to contribute to future health needs and at greatest risk could hardly be more urgent or significant. Botanical collections contribute to addressing the grand societal challenge of improving health through supporting natural product drug discovery efforts in a variety of ways.

The Role of Natural History Collections in the 21st Century

Historically, plant systematics and herbaria have roots in the first herbals or pharmacopoeias, catalogues of medicinal plants with names and descriptions of the plants and their uses, often richly illustrated (Anderson 1977; Friis 2017). Until the 17th century, botany and medicine were largely integrated sciences and plants were primarily categorized based on their uses. Traditional herbal medicine is probably as old as mankind, passed on through oral tradition and later through written texts (Leonti *et al.* 2015). Early herbarium collections were in the form of preserved specimens mounted on sheets bound together in a book for reference and teaching (Nesbitt 2014; Ahmed & Hasan 2016). Classical herbals such as Dioscorides' *De Materia Medica*, written between 40 and 90 Current Era, included around 500 medicinal plants and influenced European medicine for a thousand years (De Vos 2010). Currently, about 13,500 plant species are recorded in the Medicinal Plant Names Services, a global resource for medicinal plant names, assembled by the Royal Botanic Gardens, Kew (Medicinal Plant Names Services 2016). However, the estimated number of higher plant species alone used worldwide for medicinal purposes is more than 50,000 (Schippmann *et al.* 2002). The history of botanical collections, both living and preserved, is rooted in the use of plants for medicines and other purposes, and also today, and in the future, collections are essential for medicinal plant research and have the potential to significantly impact modern drug lead discovery. Collections are, at the very least, needed to allow authoritative identification and documentation of medicinal plant material. Additionally, collections provide a powerful framework for understanding variation of natural products at all scales from ecological or chemical types within species to chemical diversity within lineages and across the entire plant domain. In the era of Big Data and genomics, botanists of the 21st century can set the agenda by taking advantage of collections, collaboration, and an interdisciplinary approach to help develop new understanding, tools, better medicines and policies for sustainable and ethically responsible biodiversity use.

The Importance of Collections in Natural Products Research

Collections are repositories and reference for identification

A specimen deposited in a recognised herbarium serves as documentation of the identity of plant material used for natural products research and allows for later confirmation of authoritative identity of the material (Hedberg 1993; Bussmann 2015). However, herbaria are not only repositories of vouchers, but they also contain reference specimens and type specimens that can be used to verify the identity of newly collected plant material or material received from collaborators or commercial suppliers. Authoritative and verifiable identification ensures that substantial amounts of time and money are not invested in investigating plant material that may later turn out to be a different and inactive species - or may be active but cannot be identified due to lack of a voucher specimen. This may have been a real shortcoming of natural product research in past decades. An early survey by Farnsworth and Bingel (1977) concluded that out of 2399 novel chemical compounds reported in the scientific literature in 1975, only 160 of them indicated voucher specimens for future reference. The situation has improved considerably and voucher specimens are now required by journals, but the example illustrates the

historical gap between natural product research and botanical methods and expertise.

Natural product researchers should consider the importance of providing vouchers of high quality with informative labels deposited in herbaria, enabling them to be more useful for other studies (Bussmann 2015). At the same time, some herbaria today are reluctant to accept sterile or other hard to identify material as well as cultivated material. However, a sterile voucher is, in any case, better than no voucher, and even the use of material from living collections in botanical gardens need vouchering rather than just reference to an accession number in the living collection. Whereas DNA barcoding inherently will allow reliable identification of sterile and other difficult to identify material in the future, the quality of accompanying information provided on the labels as well as in the materials description in the scientific publications cannot easily be improved or retraced retrospectively. Researchers should, therefore, consider including as much information as possible directly on the labels of the voucher specimens allowing specimens to be of use for future reference as well as for other types of studies.

Collections are a representation of biodiversity

Collections in herbaria and botanical gardens have been assembled over centuries and often contain a good representation of all plant families and most genera. For natural product and bioactivity screening, collections are therefore an invaluable resource of authentically identified material allowing selection and study design to maximize taxonomic diversity or diversity with respect to habitat, life form or other specific traits. However, destructive sampling is always problematic, but in larger collections, there are often several specimens to choose between, and often loose material to be found in envelopes on some of the specimens or duplicates of accessions of plants in other preserved or living collections. Furthermore, modern hyphenated analytical methods and bioactivity screens require only small amounts of material (100–1000 mg of dried plant material) making highly informative screenings from collections possible without compromising the collections significantly although not all chemical markers are well preserved in dried material (Kongstad *et al.* 2014; Okutan *et al.* 2014; Liu *et al.* 2015). Researchers must also consider any potential effect of chemicals sometimes used for preserving specimens and, if relevant, avoid such specimens. Needless to say, permission to remove any part of a specimen in a collection must always be sought from the herbarium curator.

However, selection of plant material for screening studies should also consider any known information from traditional medicine or previous studies indicating which plants are most relevant to screen. The emerging approach of phylogenetic selection is discussed below. Traditional uses are sometimes recorded on the labels of the specimens or may be found in literature or through databases such as the NAPRALERT® database of natural products (*www.napralert.org*) maintained by the University of Illinois, Chicago. For DNA barcoding, studies relevant to authentication of medicinal plants are discussed below, 10–20 mg dried plant material is sufficient (Saslis-Lagoudakis *et al.* 2015a). In addition to using herbarium specimens, other types of collections such as seeds (which often have better preserved DNA) (Fordyce *et al.* 2013; da Fonseca *et al.* 2015), or wood collections (which often allow for sampling larger quantities), may be considered for some studies.

Collections are a source of natural variation

Documented sourcing of material of consistent quality is required as part of the regulatory guidelines for herbal products (Council of Europe 2015), yet within-species variation is a largely neglected problem in natural product research today. It is well known that the same species may express different amounts or combinations of compounds dependent on seasonality, age, geographical origin, habitat use or other variables (Gatehouse 2002; Agrawal & Fishbein 2006; Moore *et al.* 2014). Specialized compounds are not continuously expressed, but may be produced as a response to herbivory or other damage and stress in-

cluding the environment. However, most screening studies for activity only include one representative per species and subsequent identification of active compounds usually does not revisit intraspecific variation. Whereas it may not be feasible to consider intra-specific variation in large scale screening studies focusing on inter-specific diversity, collections are easily available and well-documented resources for studying both quantitative and qualitative chemical diversity within species (Berkov *et al.* 2004; Yilmaz *et al.* 2012; Saslis-Lagoudakis *et al.* 2015a). Such chemical diversity screens may subsequently help selection of the best starting material for further bioactivity studies, or hyphenated techniques may be used directly as discussed above. However, many natural products may be broken down over time compromising the value of dried and older collections for general screening.

Collections provide a window into the past

Collections are of age. They provide time referenced data points which can be used to document domestication (da Fonseca *et al.* 2015), changes in distribution associated with climate (Calinger 2015), or human interaction (Dodd *et al.* 2015; Martin *et al.* 2014), flowering times (Davis *et al.* 2015; Munson & Sher 2015; Park & Schwartz 2015), or other morphological traits (Dalrymple *et al.* 2015; Everill *et al.* 2014). Using collections to look into the past can also document the history of plant pathogens (Yoshida *et al.* 2014) such as the Irish potato famine pathogen (Martin *et al.* 2014; Yoshida *et al.* 2014), mildew (Choi & Thines 2015) or rust fungi (Braithwaite *et al.* 2009; Haudenshield & Hartman 2015). Time referenced collections may also be used for exploring chemical variation over time. Whereas records in time can be readily used and morphological traits can be measured, obtaining DNA sequence data from historical herbarium samples has been difficult using classical DNA extractions and sequencing techniques as elaborated on in relation to authentication of herbal products discussed below. However, the rapid development of so-called ancient DNA techniques (Willerslev & Cooper 2005; Sarkissian *et al.* 2015) continues to open up new opportunities for including significantly older historical samples in evolutionary studies as exemplified by several centuries old rag-weed samples (Martin *et al.* 2014) and 700 year old maize kernels (da Fonseca *et al.* 2015). Surviving plant DNA has even been retrieved from more than 20,000 years old lake sediments from Greenland (Parducci *et al.* 2012).

Examples of Collection-based Natural Product Research

Authentication of herbal products

The safety of medicinal plant use is compromised by alteration and substitution, and by the availability of prohibited plants or restricted species, which can lead to severe side effects due to the presence of toxic compounds (Gilbert 2011) or raise conservation concerns. Authentication of the qualitative and quantitative composition of herbal products is regulated by international and national guidelines such as the European Pharmacopoeia (Council of Europe 2015), which provides a series of monographs for quality control of herbal products, including recommended tests for identification. However, macroscopic or microscopic identification of plant species requires considerable expertise to differentiate between closely related or similar looking species. Furthermore, morphological characters may be indistinguishable in bulk, pulverised or otherwise processed material (Han *et al.* 2013; Kool *et al.* 2012; de Boer *et al.* 2014). Authentication therefore normally includes chemical tests, typically simple chromatographic assays, which can be applied to crude drug samples in pulverized form to verify specific chemical profiles. Chromatographic techniques may also be used to reveal adulteration as demonstrated for the Ashoka bark (*Saraca asoca* (Roxb.) Willd.), which is used in Ayurvedic medicine (Beena & Radhakrishnan 2012). However, based on chemical profiles that may not be unique to a species and may be compromised by intraspecific variation, such assays rely on the existence of well-defined chemical profiles of possible adulterants for comparison

and may not always confidently verify the botanical identity of the sample.

DNA-based identification methods or barcoding have often revealed adulteration in traditional medicinal preparations and herbal products (de Boer *et al.* 2015). For example, potentially toxic *Ephedra* L. and *Asarum* L. material was found in traditional Chinese medicinal products administered in Australia (Coghlan *et al.* 2012), and several adulterant plant species were found in herbal products from North America (Newmaster *et al.* 2013). However, DNA barcoding also has limitations. Depending on the condition of the plant material, amplification of the target DNA marker may not be practically possible. In a study including 100 museum medicinal specimens and herbal products from 92 species representing five orders, Han *et al.* (2013) were able to recover ITS2 from 90% of the museum specimens, suggesting ITS2 as a minibarcode to effectively identify species in a wide variety of specimens and medicinal materials. DNA barcodes may also lack interspecific variability, particularly among closely related species. Finally, because DNA barcoding relies on the presence of a reference database, the absence of a species from the database will impede its identification success (Stoeckle *et al.* 2011; Saslis-Lagoudakis *et al.* 2015a).

The common horsetail, *Equisetum arvense* L., is used in numerous herbal products for mild urinary and renal conditions and as skin, hair and nail remedies, but it can be adulterated with closely related species, especially *Equisetum palustre* L. that produce toxic alkaloids. The potential of using DNA barcoding for identifying *Equisetum* L. species using material from herbarium collections and commercial herbal products was tested by Saslis-Lagoudakis *et al.* (2015a). Using herbarium collections from herbarium C, it was possible to include in this study all 15 species of Equisetum and a broad geographical representation of both *Equisetum arvense* and *Equisetum palustre*. This study showed that DNA barcoding could be used for authentication of *Equisetum arvense* products, and that collections can provide easily accessible high quality samples for creating barcoding reference libraries (Saslis-Lagoudakis *et al.* 2015a). Other examples are provided by market surveys of the drug trade of *Phyllanthus* in India (Srirama *et al.* 2010) and a broad spectrum of medicinal plants traded in Morocco (Kool *et al.* 2012).

Looking ahead, phylogeny and comparative sequence analysis made possibly by sampling collections, opens up the possibility of enhanced regulatory control. Additional opportunities could be tracking the supply chain to elucidate the drivers and the extent of substitution and adulteration – data that could provide significantly more effective monitoring to protect health of consumers on one hand, and health of the wild biodiversity resource on the other hand.

Phylogenetic selection of medicinal plants and new leads

During evolution, plants and other organisms have developed a diversity of chemical defense compounds leading to the evolution of various groups of specialized metabolites, such as alkaloids, terpenoids, and phenolics, selected for their endogenous defense function (Ehrlich & Raven 1964; Becerra 1997). Intuitively, a correlation between phylogeny and biosynthetic pathways is sometimes assumed (Ehrlich & Raven 1964; Hegnauer 1962–1973) and could offer a predictive approach enabling the elucidation of biosynthetic pathways (Rodman *et al.* 1998; Rønsted *et al.* 2003), insights into defense against herbivores (Wink & Mohamed 2003; Becerra *et al.* 2009), more efficient selection of plants for the development of traditional medicine and lead discovery (Rønsted *et al.* 2008, 2012; Zhu *et al.* 2011; Grace *et al.* 2015; Saslis-Lagoudakis *et al.* 2015b) as well as inform conservation policies (Forest *et al.* 2007).

How can we implement a phylogenetic selection of medicinal plants? One approach may be to identify 'Hot Nodes' of bioactivity. By exploring plant uses in a phylogenetic context, based on plant molecular phylogenies that were generated largely from herbarium collections, Saslis-Lagoudakis *et al.* (2011, 2012) demonstrated that certain nodes are significantly overrepresented by species with different medicinal properties.

Another approach is to better understand the correlation between phylogeny, chemistry and bioactivi-

ty. Alkaloids occurring in Amaryllidaceae subfamily Amaryllidoideae are known to possess central nervous system activities (Jin 2011) including galanthamine originally isolated from snowdrops (genus *Galanthus* L.), which is registered as a drug for the inhibition of acetylcholinesterase associated with the progression of Alzheimers disease (Heinrich & Theo 2004). Taking advantage of collections, Rønsted *et al.* (2008, 2012), explored the phylogenetic correlation of alkaloids with central nervous system activities in Amaryllidaceae subfamily Amaryllidoideae. They found significant correlation of alkaloid diversity and in vitro inhibition of acetylcholinesterase and binding to the serotonin reuptake transporter, but the effect was not strong.

Phylogenetic studies can also provide insights into the origins and explanations of the uses of medicinal plants. *Aloe vera* L. supports a substantial global trade, but both its natural origin and explanations for its popularity over 500 related *Aloe* species in one of the world's largest succulent groups, have remained uncertain. Comparison of monosaccharide profiles of 30 species representing the diversity of aloes (Grace *et al.* 2013) found the common glucose-mannose-xylose profile identified in *Aloe vera* and other commercially important species, to be shared by many other *Aloe* species. Using a phylogenetic approach and published medicinal uses, Grace *et al.* (2015) constructed a phylogenetic hypothesis including over 200 species from a combination of curated living collections and wild origins. They found that medicinal use was correlated with the phylogeny and succulence of the leaves, and for the first time, the origin of *Aloe vera* was traced to the Arabian Peninsula, suggesting a connection with ancient trade routes as an explanation for the global popularity of *Aloe vera* today.

With almost 2000 species and only about 5% of species in the genus chemically investigated (Vasas & Hohman 2014), *Euphorbia* exemplifies the need for a systematic approach to plant-based drug discovery an effort currently being undertaken as part of the MedPlant International Training programme (Ernst *et al.* 2015). The genus *Euphorbia* (spurges, Euphorbiaceae) is the third largest genus of flowering plants, with a near-cosmopolitan distribution and remarkable morphological diversity, including annual herbs, succulents and large trees, united by a unique, flower-like inflorescence and often poisonous, milky latex. Medicinal uses have been identified for >5% of the species in the genus (Ernst *et al.* 2015) and ingenol mebutate (Picato®), a diterpenoid isolated from *Euphorbia peplus* L. is marketed for the topical treatment of actinic keratosis (Berman 2012). Given the high number of chemically unexplored species, and the signature diterpenoid chemistry of *Euphorbia* latex (Vasas & Hohman 2014), species with a higher production of compounds of interest or new drug candidates with therapeutically relevant activity profiles await discovery.

Despite these few examples, the predictive power of phylogenies is still not fully explored, and there are no standard methods for application of phylogenetic selection (Saslis-Lagoudakis *et al.* 2015b). Development of new approaches and technologies for selection of biodiversity resources for lead discovery is also one of the objectives of the MedPlant International Training Network, *www.MedPlant.eu*, which aims at training a new generation of young scientists in interdisciplinary approaches to explore medicinal plant diversity.

Future Directions

An interdisciplinary approach

Science today has become a collaborative and highly interdisciplinary effort, where scientists work together and take advantage of highly specialised complementary expertise to gain as much information as possible from their data (Van Noorden 2015). Such interdisciplinary science is not only necessary because the amount and types of data we can obtain continues to increase, but also because new exciting research questions may be addressed. To solve the grand challenges facing society today – energy, water, climate, food, and health – scientists and social scientists must work together (Ledford 2015).

The MedPlant programme synthesizes and takes advantage of botany, phylogeny, bioinformatics, eth-

nobotany, natural products chemistry and bioactivity studies to take a fresh look at the evolution of chemical diversity, the development of pharmacopoeias, and sustainability and safety, to develop and refine new approaches and technologies for selection of biodiversity resources for lead discovery. However, interdisciplinarity takes time and requires a mutual mission and the will to understand and overcome different paradigms, theoretical, and methodological traditions (Ledford 2015; Van Noorden 2015).

Collections are at the heart of these interdisciplinary efforts by providing material and data, which may be used for addressing a plethora of research questions. Botanists and curators working with collections are at an advantage by knowing the collections and being able to define exciting new research questions that can be addressed with the collections and by joining forces with relevant colleagues in other scientific fields such as ecology, genetics, bioinformatics, history, ethnopharmacology, natural products research, as well as with governmental agencies, NGOs, or industry as relevant. In the era of Big Data, botanists and curators of collections also have an important role in securing high quality data for interdisciplinary studies, including the curation of the ever growing public databases (Maldonado *et al.* 2015).

Merging collections and archives

The value of collections is related to the associated information, such as the name of the collector, the date, locality and recorded field data. Additional information may be accompanying collections, such as expedition journals, letters and lists from the collector, card catalogues, field images of plants and scientific publications. However, a clear link between the collections and scientific publications or archives is not always present, but may be retrieved through additional research. A classical example is the lack of assigned type specimens to species named by Linnaeus. The Linnaean Plant Name Typification Project is now addressing this by establishing type specimens retroactively for the 9000 plant names of species established and named by Linnaeus, so that the names can be correctly used (*http://www.nhm.ac.uk/our-science/data/linnaean-typification/*; accessed 16/1-2016).

The possibility of obtaining DNA sequences from herbarium specimens also allows for better identification of specimens that are difficult to identify either because of the quality or incompleteness of the specimen or because of uncertain species concepts or complexes. Thapsigargin from the Mediterranean *Thapsia garganica* L. is currently being developed into a product for treatment of certain cancer forms, and alternative production methods are being investigated to overcome expected supply problems (Andersen *et al.* 2015). However, the biosynthetic pathway to thapsigargin and the species concepts in the genus *Thapsia* L. are not well understood, impeding prediction of a more productive better source for the production of thapsigargin. In a phylogenetic study of *Thapsia* (Weitzel *et al.* 2014), it was possible to link published chemical screening data (Rasmussen *et al.* 1981; Christensen *et al.* 1997) with the original voucher specimens, thereby allowing reassessment of the original identifications in a difficult plant genus. Reassessing published chemical distribution data in a phylogenetic context allows us to both improve our chemotaxonomic understanding and to evaluate the taxonomic value of chemical markers, as well as to take advantage of the published data to predict biosynthetic pathways or select clades of interest for further chemical studies (Rønsted *et al.* 2008; Larsson & Rønsted 2014; Weitzel *et al.* 2014). Linking specimens with archives or published data improve the value of collections but also of the aforementioned, or even left-over material or other biocultural collections (Hedberg 1993; Salick *et al.* 2014; Maldonado *et al.* 2015; Soelberg *et al.* 2015).

Soelberg *et al.* (2015) discovered archived historical documents from the colonial days of Ghana, describing medicinal plant uses among the Fante, Ga and Ashanti people of present-day Ghana. These historical medicinal uses could be linked to original botanical specimens in European herbaria and provided a unique opportunity to gain insight to the historical Materia Medica of Ghana. By comparison to contemporary medicinal plant uses, this study provided the

foundation to reconstruct forgotten medicines, i.e. lost or discontinued Ghanaian plant uses in local or ethnopharmacological contexts. The scientifically strong voucher material allowed for authoritative identification of a high number of historical medicinal plants and their roots in traditional Ghanaian medicine systems 200-300 years ago. Of the 134 specific historical uses, 41 (31%) were traced to contemporary medicinal plant uses in Ghana and represent some of the most important Ghanaian medicinal plant species. However, 93 (69%) of the historical uses could not be traced and appear to have been discontinued or forgotten. Among the Ga people, only two medicinal plants species have become rare or locally extinct, thus the vast majority of the loss of knowledge appears to be due to cultural extinction. This conclusion confirms current awareness that traditional languages and practices and thus knowledge about how to use the plants may be disappearing faster than the plants themselves (e.g., Alves & Rosa 2007).

Aligning with international regulations

Along with the potential discovery of new medicinal uses of plant species based on collections, two potential problems related to the conservation and intellectual property rights arise. When paclitaxel (taxol) from the Pacific yew tree, *Taxus brevifolia* Nutt., was discovered as a cure against various forms of cancer in 1962 through a large screening programme conducted by the National Cancer Institute (Wani *et al.* 1971), the pacific yew tree was already becoming threatened, but a more stable resource of the drug was secured through chemical semi-synthesis from the common yew, *Taxus baccata* L. (Malik *et al.* 2011). However, a survey by the Botanic Gardens Conservation International (Hawkins 2008), warned that 'cures for things such as cancer and HIV may become extinct before they are ever found'. They identified 400 medicinal plants at risk of extinction from over-collection and deforestation. A recent example is *Hoodia gordonii* (Masson) Sweet ex Decne from Namibia and South Africa, which became threatened by collectors after it was advertised as a potential source of weight loss drugs (Vermaak *et al.* 2011).

Whereas taxol was discovered through a random screening programme, the benefits of *Hoodia* was based on the San peoples' use of this plant, but without seeking prior informed consent from the San (Vermaak *et al.* 2011). Since the 1980s, the use of biological resources and indigenous peoples knowledge has been addressed by international conventions, such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; www.CITES.org) and The Convention on Biological Diversity (CBD; *www.cbd.int*) and the recent addition, the Nagoya Protocol (https://www.cbd.int/abs/doc/protocol/nagoya-protocol-en.pdf).

Researchers working on drug discovery from collections must, therefore, be aware of international regulations, as well as consider solutions to potential supply problems and conservations issues. Embracing and consolidating strong North-South collaborations is part of a forward-looking solution. Doing so, botanists of the 21st century can set the agenda by taking advantage of collections, collaboration, and an interdisciplinary approach to help develop new understanding, tools, better medicines and policies for sustainable and ethically responsible use of biodiversity resources.

Medicinal plant research can also benefit collections

Although the prospect of developing new drugs improving human health is in itself worthwhile, medicinal plant or natural products research can also benefit collections, through increased public awareness and appreciation of both biodiversity and the importance of collections – if facilitated through public dissemination and engagement activities. Collection-based drug discovery programs, big or small, may also help raise funds for taxonomic and curatorial work in connection with medicinal plant research projects. New collections or fieldwork may also provide new specimens to the collections and other additional information or samples can be collected simultaneously for uses other than drug discovery (e.g., Maldonado *et al.*

2015). Collections have their roots in herbals and finding new exciting ways of integrating collections with modern drug discovery, through interdisciplinary collaborations, is highly timely and will likely provide new synergy and results benefitting both our collections and our health.

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References

Agrawal, A.A. & Fishbein, M. (2006). Plant defense syndromes. *Ecology* 87: S132-S149.

Ahmed, S.A. & Hasan, M.M. (2016). Importance of herbaria in herbal drug discovery. *World Journal of Pharmaceutical Sciences* 4: 127-129.

Alves, R.R.N. & Rosa, I.M.L. (2007). Biodiversity, traditional medicine and public health: Where do they meet? *Journal of Ethnobiology and Ethnomedicine* 3: 14.

Andersen, T.B., López, C.Q., Manczak, T., Martinez, K. & Simonsen, H.T. (2015). Thapsigargin – from *Thapsia* L. to mipsagargin. *Molecules* 20: 6113-6127.

Anderson, F.J. (1977). *An Illustrated History of the Herbals.* Columbia University Press, New York.

Becerra, J.X. (1997). Insects on plants: Macroevolutionary chemical trends in host use. *Science* 276: 253-256.

Becerra, J.X., Noge, K. & Venable, D.L. (2009). Macroevolutionary chemical escalation in an ancient plant-herbivore arms race. *Proceedings of the National Academy of Sciences. USA.* 106: 18062-18066.

Beena, C. & Radhakrishnan, V.V. (2012). Quality assessment evaluation of the market samples of important ayurvedic drug asoka bark. *Annals of Phytomedicine* 1: 95-98.

Berkov, S., Sidjimova, B., Evstatieva, L. & Popov, S. (2004). Intra-specific variability in the alkaloid metabolism of *Galanthus elwesii*. *Phytochemistry* 65: 579-586.

Berman, B. (2012). New developments in the treatment of actinic keratosis: Focus on ingenol mebutate gel. *Clinical, Cosmetic and Investigational Dermatology* 5: 111-122.

Bohlin, L., Alsmark, C., Göransson, U., Klum, M., Wedén, C. & Backlund, A. (2012). Strategies and methods for a sustainable search for bioactive compounds. *In:* C. Tringali (ed.), *Bioactive Compounds from Natural Sources; Natural Products as Lead Compounds in Drug Discovery.* (Chapter 1). CRC Press, Boca Raton, London, New York. Pp. 1-36.

Braithwaite, K.S., Croft, B.J., Magarey, R.C. & Scharaschkin, T. (2009). Phylogenetic placement of the sugarcane orange rust pathogen *Puccinia kuehnii* in a historical and regional context. *Australasian Plant Pathology* 38: 380-388.

Bussmann, R.W. (2015). Taxonomy - An irreplaceable tool for validation of herbal medicine. *In:* P. K. Mukherjee (ed.), *Evidence-based Validation of Herbal Medicines.* (Chapter 4). Elsevier, Amsterdam. Pp. 87-118.

Calinger, K.M. (2015). A functional group analysis of change in the abundance and distribution of 207 plant species across 115 years in north-central North America. *Biodiversity and Conservation* 24: 2439-2457.

Christensen, S.B., Andersen, A. & Smitt, U.W. (1997). Sesquiterpenoids from *Thapsia* species and medicinal chemistry of the thapsigargins. *Fortschritte der Chemie organischer Naturstoffe* 71: 129-167.

Choi, Y.J. & Thines, M. (2015). Host jumps and radiation, not co-divergence drives diversification of obligate pathogens. A case study in Downy Mildews and Asteraceae. *PLoS ONE* 10: e0133655.

Coghlan, M.L., Haile, J., Houston, J., Murray, D.C., White, N.E., Moolhuijzen, P., Bellgard, M.I. & Bunce, M. (2012). Deep sequencing of plant and animal DNA contained within traditional chinese medicines reveals legality issues and health safety concerns. *PLoS Genetics* 8: e1002657.

Council of Europe (2015). *European Pharmacopoeia.* 8th Edition. https://www.edqm.eu/en/european-pharmacopoeia-8th-edition-1563.html

Cragg, G.M. & Newman, D.J. (2009). Nature: A vital source of leads for anticancer drug development. *Phytochemistry Reviews* 8: 313-331.

Cragg, G.M. & Newman, D.J. (2013). Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta-General Subjects* 1830: 3670-3695.

da Fonseca, R.R., Smith, B.D., Wales, N., Cappellini, E., Skoglund, P., Fumagalli, M., Samaniego, J.A, Carore, C., Avila-Arcos, M.C., Hufnagel, D.E., Korneliussen, T.S., Viera, F.G., Jakobssen, M., Arriaza, B., Willerslev, E., Nielsen, R., Hufford, M.B., Albrechtsen, A., Ross-Ibarra, J. & Gilbert, M.T.P. (2015). The origin and evolution of maize in the southwestern United States. *Nature Plants* 1: Article number 14003.

Dalrymple, R.L., Buswell, J.M. & Moles, A.T. (2015). Asexual plants change just as often and just as fast as do sexual plants when introduced to a new range. *Oikos* 124: 196-205.

Davis, C.C., Willis, C.G., Connolly, B., Kelly, C. & Ellison, A. (2015). Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms. *American Journal of Botany* 102: 1599-1609.

de Boer, H.J., Ouarghidi, A., Martin, G., Abbad, A. & Kool, A. (2014). DNA barcoding reveals limited accuracy of identifications based on folk taxonomy. *PLoS ONE* 9: e84291.

de Boer, H.J., Ichim, M.C. & Newmaster, S.G. (2015). DNA Barcoding and pharmacovigilance of herbal medicines. *Drug Safety* 38: 611-620.

De Vos, P. (2010). European Materia Medica in historical texts: Longevity of a tradition and implications for future use. *Journal of Ethnopharmacology* 132: 28-47.

Dodd, A.J., Burgman, M.A., McCarthy, M.A. & Ainsworth, N. (2015). The changing patterns of plant naturalization in Australia. *Diversity and Distributions* 21: 1038-1050.

Ehrlich, P.R. & Raven, P.H. (1964). Butterflies and plants – a study in coevolution. *Evolution* 18: 586-608.

Ernst, M., Grace, O.M., Saslis-Lagoudakis, C.H., Nilsson, N., Simonsen, H.T. & Rønsted, N. (2015). Global medicinal uses of *Euphorbia* L. (Euphorbiaceae). *Journal of Ethnopharmacology* 176: 90-101.

Everill, P.H., Primack, R.B., Ellwood, E.R. & Melaas, E.K. (2014). Determining past leaf-out times of New England's deciduous forests from herbarium specimens. *American Journal of Botany* 101: 1293-1300.

Farnsworth, N.R. & Bingel, A.S. (1977). Problems and prospects of discovering new drugs from higher plants by pharmacological screening. *In:* H. Wagner & P. Wolff (eds.), *Medicinal Plant Research*. Springer Verlag, Berlin. Pp. 1-22.

Fordyce, S.L., Avila-Arcos, M.C., Rasmussen, M., Cappellini, E., Romero-Navarro, J.A., Wales, N., Alquezar-Planas, D.E., Penfield, S., Brown, T.A., Vielle-Calzada, J.P., Montiel, R., Jorgensen, T., Odegaard, N., Jacobs, M., Arriaza, B., Higham, T.F.G., Ramsey, C.B., Willerslev, E. & Gilbert, M.T.P. (2013). Deep sequencing of RNA from ancient maize kernels. *PLoS One* 8: e50961.

Forest, F., Grenyer, R., Rouget, M., Davies, T.J., Cowling, R.M., Faith, D.P., Balmford, A., Manning, J.C., Proche, S., van der Bank, M., Reeves, G., Hedderson, T.A.J. & Savolainen, V. (2007). Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature* 445: 757-760.

Friis, I. (2017). Temperate and tropical collections: The changing species concept and other ideas behind their development. *Scientia Danica, B (Biologica)* 6: 15-38.

Gatehouse, J. (2002). Plant resistance towards insect herbivores: A dynamic interaction. *New Phytologist* 156: 145-168.

Gilbert, N. (2011). Regulations: Herbal medicine rule book. *Nature* 480: S98-S99.

Grace, O.M., Dzajic, A., Jäger, A.K., Nyberg, N.T., Onder, A. & Rønsted, N. (2013). Monosaccharide analysis of succulent leaf tissue in *Aloe*. *Phytochemistry* 93: 79-87.

Grace, O.M., Buerki, S., Symonds, M.R.E., Forest, F., van Wyk, A.E., Smith, G.F., Klopper, R.R., Bjora, C.S., Neale, S., Sebsebe Demissew, Simmonds, M.S.J. & Rønsted, N. (2015). Evolutionary history and leaf succulence as explanations for medicinal use in aloes and the global popularity of *Aloe vera*. *BMC Evolutionary Biology* 15: 29.

Han, J., Zhu, Y., Chen, X., Liao, B., Yao, H., Song, J., Chen, S. & Meng, F. (2013). The short ITS2 sequence serves as an efficient taxonomic sequence tag in comparison with the full-length ITS. *BioMed Research International* 2013: Article ID 741476.

Haudenshield, J.S. & Hartman, G.S. (2015). Archaeophytopathology of *Phakopsora pachyrhizi*, the soybean rust pathogen. *Plant Disease* 99: 575-579.

Hawkins, B. (2008). *Plants for Life: Medicinal Plant Conservation and Botanic Gardens*. Botanic Gardens Conservation International, Kew, UK.

Hedberg, I. (1993). Botanical methods in ethnopharmacology and the need for conservation of medicinal plants. *Journal of Ethnopharmacology* 38: 121-128.

Hegnauer, R. (1962–1973). Chemotaxonomie der Pflanzen. Vol. 1-6. Birkhäuser Verlag, Basel.

Heinrich, M. & Teoh, H.L. (2004). Galanthamine from snowdrop – the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *Journal of Ethnopharmacology* 92: 147-162.

Jin, Z. (2011). Amaryllidaceae and Sceletium alkaloids. *Natural Product Reports* 28: 1126-1142.

Kongstad, K.T., Wubsheet, S.G., Johannesen, A., Kjellerup, L., Winther, A.M.L., Jäger, A.K. & Staerk, D. (2014). High-resolution screening combined with HPLC-HRMS-SPE-NMR for identification of fungal plasma membrane H+-ATPase inhibitors from plants. *Journal of Agricultural and Food Chemistry* 62: 5595-5602.

Kool, A., Krüger, Å., Rydberg, A., Abbad, A., Björk, L. & Martin, G. (2012). Molecular identification of commer-

cialized medicinal plants in southern Morocco. *PLoS ONE* 7: e39459.

Larsson, J., Gottfries, J., Muresan, S. & Backlund, A. (2007). ChemGPS-NP: Tuned for navigation in biologically relevant chemical space. *Journal of Natural Products* 70: 789-794.

Larsson, S. & Rønsted, N. (2014). Reviewing Colchicaceae alkaloids – Perspectives of evolution on medicinal chemistry. *Current Topics in Medicinal Chemistry* 14: 274-289.

Ledford, H. (2015). How to solve the world's biggest problems. *Nature* 525 (7569): 308-311.

Leonti, M., Staub, P.O., Cabras, S., Castellanos, M.E. & Casu, L. (2015). From cumulative cultural transmission to evidence-based medicine: Evolution of medicinal plant knowledge in southern Italy. *Frontiers in Pharmacology* 6: 207.

Liu, Y.Q., Staerk, D., Nielsen, M.N., Nyberg, N. & Jäger, A.K. (2015). High-resolution hyaluronidase inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of anti-necrosis constituents in Chinese plants used to treat snakebite. *Phytochemistry* 119: 62-69.

Maldonado, C., Molina, C., Zizka, A., Persson, C., Alban, J., Chilquillo, E., Rønsted, N. & Antonelli, A. (2015). Estimating species diversity and distribution in the era of Big Data: To what extent can we trust public databases? *Global Ecology and Biogeography* 24: 973-984.

Malik, S., Cusidó, R.M., Mirjalili, M.H., Moyano, E., Palazón, J. & Bonfill, M. (2011). Production of the anticancer drug taxol in *Taxus baccata* suspension cultures: A review. *Process Biochemistry* 46 (1): 23-34.

Martin, M.D., Cappellini, E., Samaniego Castruita, J.A., Zepeda Mendoza, M.L., Campos, P., Seguin-Orlando, A., Wales, N., Orlando, L.A.A., Ho, S.Y.W., Dietrich, F.S., Mieczkowski, P.A., Heitman, J., Willerslev, E., Krogh, A., Ristaino, J.B. & Gilbert, M.T.P. (2014). Reconstructing genome evolution in historic samples of the Irish potato famine pathogen. *Nature Communications* 4: 2172.

Martin, M.D., Zimmer, E.A., Olsen, M.T., Foote, A.D., Gilbert, M.T.P. & Brush, G.S. (2014). Herbarium specimens reveal a historical shift in phylogeographic structure of common ragweed during native range disturbance. *Molecular Ecology* 23: 1701-1716.

Medicinal Plant Names Services Portal. Royal Botanic Gardens, Kew (accessed on 16 January 2016).

Moore, B., Andrew, R.L., Kulheim, C. & Foley, W.J. (2014). Explaining intraspecific diversity in plant secondary metabolites in an ecological context. *New Phytologist* 201: 733-750.

Munson, S.M. & Sher, A.A. (2015). Long-term shifts in the phenology of rare and endemic Rocky Mountain plants. *American Journal of Botany* 102: 1268-1276.

Nesbitt, M. (2014). Use of herbarium specimens in ethnobotany. *In:* J. Salick, K. Konchar & M. Nesbitt (eds.), *Curating Biocultural Collections*. Royal Botanic Gardens, Kew. Pp. 313-328.

Newman, D.J. & Cragg, G.M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products* 75: 311-335.

Newmaster, S., Grguric, M., Shanmughanandhan, D., Ramalingam, S. & Ragupathy, S. (2013). DNA barcoding detects contamination and substitution in North American herbal products. *BMC Medicine* 11: 222.

Okutan, L., Kongstad, K.T., Jäger, A.K. & Staerk, D. (2014). High-resolution alpha-Amylase assay combined with High-Performance Liquid Chromatography Solid-Phase Extraction-Nuclear Magnetic Resonance Spectroscopy for expedited identification of alpha-Amylase inhibitors: Proof of concept and alpha-Amylase inhibitor in Cinnamon. *Journal of Agricultural and Food Chemistry* 62: 11465-11471.

Parducci, L., Jørgensen, T., Tollefsrud, M.M., Elverland, E., Alm, T., Fontana, S.L., Bennett, K.D., Haile, J., Matetovici, I., Suyama, Y., Edwards, M.E., Andersen, K., Rasmussen, M., Boessenkool, S., Coissac, E., Brochmann, C., Taberlet, P., Houmark-Nielsen, M., Krog Larsen, N., Orlando, L., Gilbert, M.T.P, Kjær, K.H., Alsos, I.G. & Willerslev, E. (2012). Glacial survival of boreal trees in northern Scandinavia. *Science* 335: 1083-1086.

Park, I.W. & Schwartz, M.D. (2015). Long-term herbarium records reveal temperature-dependent changes in flowering phenology in the southeastern USA. *International Journal of Biometeorology* 59: 347-355.

Rasmussen, U., Christensen, S.B. & Sandberg, F. (1981). Phytochemistry of the genus *Thapsia*. *Planta Medica* 43: 336-341.

Rodman, J.E., Soltis, P.S., Soltis, D.E., Sytsma, K.J. & Karol, K.G. (1998). Parallel evolution of glucosinolate biosynthesis inferred from congruent nuclear and plastid gene phylogenies. *American Journal of Botany* 85: 997-1006.

Rønsted, N., Franzyk, H., Mølgaard, P., Jaroszewski, J.W. & Jensen, S.R. (2003). Chemotaxonomy and evolution of *Plantago*. *Plant Systematics and Evolution* 242: 63-82.

Rønsted, N., Savolainen, V., Mølgaard, P. & Jäger, A.K. (2008). Phylogenetic selection of *Narcissus* species for drug discovery. *Biochemical Systematics and Ecology* 36: 417-422.

Rønsted, N., Symonds, M.R.E., Birkholm, T., Brøgger Christensen, S., Meerow, A.W., Molander, M., Mølgaard, P., Petersen, G., Rasmussen, N., van Staden, J., Stafford, G.I. & Jäger, A.K. (2012). Can phylogeny predict chemical diversity and potential medicinal activity of plants? A case study of Amaryllidaceae. *BMC Evolutionary Biology* 12: 182.

Salick, J., Konchar, K. & Nesbitt, M. (eds.) (2014). *Curating Biocultural Collections: A Handbook*. Royal Botanic Gardens, Kew.

Sarkissian, C.D., Allentoft, M.E., Avila-Arcos, M.C., Barnett, R., Campos, P.F., Cappellini, E., Ermini, L., Fernandez, R., da Fonseca, R., Ginolhac, A., Hansen, A.J., Jonsson, H., Korneliussen, T., Margaryan, A., Martin, M.D., Moreno-Mayar, J.V., Raghavan, M., Rasmussen, M., Velasco, M.S., Schroeder, H., Schubert, M., Seguin-Orlando, A., Wales, N., Gilbert, M.T.P., Willerslev, E. & Orlando, L. (2015). Ancient genomics. *Philosophical Transactions of the Royal Society B - Biological Sciences* 370 (1660): Article Number UNSP 20130387.

Saslis-Lagoudakis, C.H., Klitgaard, B.B., Forest, F., Francis, L., Savolainen, V., Williamson, E.M. & Hawkins, J.A. (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, C.H., Savolainen, V., Williamson, E.M., Forest, F., Wagstaff, S.J., Baral, S.R., Watson, M.F., Pendry, C.A. & Hawkins, J.A. (2012). Phylogenies reveal predictive power of traditional medicine in bioprospecting. *Proceedings of the National Academy of Sciences, U.S.A.* 109: 15835-15840.

Saslis-Lagoudakis, C.H., Iwanycki, N., Bruun-Lund, S., Petersen, G., Seberg, O., Jäger, A.K. & Rønsted, N. (2015a). Identification of common horsetail (*Equisetum arvense* L. Equisetaceae), using Thin Layer Chromatography versus DNA barcoding. *Scientific Reports* 5: 11942.

Saslis-Lagoudakis, C.H., Rønsted, N., Clarke, A.C. & Hawkins, J.A. (2015b). Evolutionary approaches to ethnobiology. *In:* U.P. Albuquerque, P.M. De Medeiros & A. Casas (eds.), *Evolutionary Ethnobiology*. Springer Publishing, Switzerland. Pp. 59-72.

Scannell, J.W., Blanckley, A., Boldon, H. & Warrington, B. (2012). Diagnosing the decline in pharmaceutical R&D efficiency. *Nature Reviews Drug Discovery* 11: 191-200.

Schippmann, U., Leaman, D.J. & Cunningham, A.B. (2002). *Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues*. FAO, Rome, Italy.

Soelberg, J., Asase, A., Akwetey, G. & Jäger, A.K. (2015). Historical versus contemporary medicinal plant uses in Ghana. *Journal of Ethnopharmacology* 160: 109-131.

Srirama, R., Senthilkumar, U., Sreejayan, N., Ravikanth, G., Gurumurthy, B.R., Shivanna, M.B., Sanjappa, M., Ganeshaiah, K.N. & Uma Shaanker, R. (2010). Assessing species admixtures in raw drug trade of *Phyllanthus*, a hepato-protective plant using molecular tools. *Journal of Ethnopharmacology* 130: 208-215.

Stoeckle, M.Y., Gamble, C.C., Kirpekar, R., Young, G., Ahmed, S. & Little, D.P. (2011). Commercial teas highlight plant DNA barcode identification successes and obstacles. *Scientific Reports* 1: Article 42.

Van Noorden, R. (2015). Interdisciplinary research by the numbers. *Nature* 525 (7569): 306-307.

Vasas, A. & Hohmann, J. (2014). *Euphorbia* diterpenes: Isolation, structure, biological activity, and synthesis (2008-2012). *Chemical Reviews* 114: 8579-8612.

Vermaak, I., Hamman, J.H. & Viljoen, A.M. (2011). *Hoodia gordonii*: An up-to-date review of a commercially important anti-obesity plant. *Planta Medica* 77(11): 1149-1160.

Wani, M, Taylor, H., Wall, M., Coggon, P. & McPhail, A. (1971). Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *Journal of the American Chemical Society* 93: 2325-2327

Weitzel C., Rønsted, N., Spalik, K. & Simonsen, H.T. (2014). Resurrecting deadly carrots. Towards a revision of *Thapsia* L. (Apiaceae) based on phylogenetic analysis of nrITS sequences and chemical profiles. *Botanical Journal of the Linnean Society* 174: 620-636.

Willerslev, E. & Cooper, A. (2005). Ancient DNA. *Proceedings of the Royal Society of London B - Biological Series* 272 (1558): 3-16.

Wink, M. & Mohamed, G.I.A. (2003). Evolution of chemical defense traits in the Leguminosae: Mapping of distribution patterns of secondary metabolites on a molecular phylogeny inferred from nucleotide sequences of the rbcL gene. *Biochemical Systematics and Ecology* 31: 897-917.

Yilmaz, A., Nyberg, N.T. & Jaroszewski, J.W. (2012). Extraction of alkaloids for NMR-based profiling: Exploratory analysis of an archaic *Cinchona* bark collection. *Planta Medica* 78: 1885-1890.

Yoshida, K., Burbano, H.A., Krause, J., Thines, M., Weigel, D. & Kamoun, S. (2014). Mining herbaria for plant pathogen genomes: Back to the future. *PLoS Pathogens* 10(4): e1004028.

Zhu, F., Qin, C., Tao, L., Liu, X., Shi, Z., Ma, X., Jia, J.,

Tan, Y., Cui, C., Lin, J., Tan, C., Jiang, Y. & Chen, Y. (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.