Munir Ozturk · Khalid Rehman Hakeem
Editors

Plant and Human Health, Volume 1

Ethnobotany and Physiology

Springer

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This volume is dedicated to

Abu Rayḥan Muḥammad ibn Aḥmad Al-Biruni

He was born in Hive-Turkmenistan in the year 973 and died on December 13, 1051, in Ghazni in Afghanistan. Al-Biruni was one of the famous scholars, who contributed much to the world of science. A book on the Medicinal Curricula "Kitab al-saydala fi al-Tıb" published by him covered details on 200 herbal drugs. Pic source: Google.com

Foreword

Her Excellency

If biodiversity underpins life on earth, then medicinal plants and traditional knowledge have underpinned the development of modern medicine. At the dawn of a new millennium, one of the most pressing challenges of our time is the continuing, and at times irreversible, loss of biodiversity and its associated precious knowledge on our planet.

Global efforts to reduce biodiversity loss had begun with the establishment of the Convention on Biological Diversity (CBD) in 1992 at the Rio Earth Summit and today the CBD has 193 parties (or governments) as members. This seminal moment has represented a dramatic step forward in the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of benefits arising from the use of genetic resources. Subsequent to this major effort, the Nagoya Convention further enshrines the need to relook at Access and Benefit Sharing.

To keep reminding the world of the urgency of preserving our biodiversity, United Nations has decreed the period 2011–2020 as the Decade on Biodiversity. However, nowhere is the need for conservation and sustainable utilization of biodiversity greater than in sub-Saharan Africa, whose biodiversity wealth is uniquely important from a global conservation viewpoint.

The African continent is home to around 60,000 plant species, of which at least 35,000 are found nowhere else. Africa's biodiversity wealth is unfortunately not uniformly distributed. Countries including Madagascar and South Africa have been classified as "megadiverse" countries and the world's 17 most biologically diverse countries that together account for nearly 70% of global species diversity.

Despite its enormous natural wealth, sub-Saharan Africa faces daunting conservation challenges. Its flora and fauna are under threats and climate change is not helping. Biodiversity loss, in Africa and in other parts of the world, has a significant impact on economic growth and social development. For the rural citizens, it has the effect of removing key sources of food, fuel, and medicines, as well as adversely affecting tourism and pharmaceuticals—from a reduction in the availability of medicinal plants. New knowledge, about conservation and whole plant utilization, is needed, not just to strengthen the conservation effort but to harness this unique patrimony of natural resources to foster economic development, reduce poverty, and protect the environment.

Seen in this context, this volume, *Plant and Human Health, Volume 1: Ethnobotany and Physiology* edited by Munir Ozturk and Khalid Rehman Hakeem, adds new contribution for celebrating traditional knowledge and how the latter has underpinned our well-being providing us with food and medicine. It will help advance our understanding of the increasingly crucial role that plants play in the economic, cultural, medical, and social spheres of our lives. This volume brings on board contributions from several continents, and it is a welcome addition in terms of safeguarding this previous knowledge for humanity as a whole.

The volume also highlights the contributions from a diverse range of scholars who offer fresh, new insights on novel sources of materials and leads including bryophytes and lichens as well as a wide range of important, related topics, including new ethnobotanical explorations that would add to the lore of knowledge for humanity now and into an uncertain future.

Sustainable utilization and management of plant genetic resources is a topic of contemporary significance. By marshaling the latest evidence and cutting-edge as well as age-old knowledge, this volume should find broad appeal among academics, scientists, farmers, policy-makers, and all those who are committed to reducing biodiversity loss on our planet and promoting new leads for the development of new drugs and other products that will sustain our well-being.

Quatre Bornes, Mauritius Ameenah Gurib-Fakim

Preface

The association of living beings on our planet originated with the beginning of life. The use of herbal products for their healing powers can be traced to earliest of myths, traditions, and writings. The plant-based medicine systems evolved primarily within local areas and produced the well-known traditional herbal treatment systems. The history of herbal use now dates back to 60,000 years, because 8 species of flowering plants have been found at the old burial site in a cave in northern Iraq—Shanidar. It is followed by the history of the use of cannabis going back to 12,000 years and olive native to Asia Minor, first domesticated in the Eastern Mediterranean between 8000 and 6000 years ago. It spread from Iran, Syria, and Palestine to the rest of the Mediterranean basin. One of the important herbs used since early times for medicinal purposes is garlic, which has been used for over 7000 years and was found in Egyptian pyramids as well as ancient Greek temples. We come across notations on garlic in medical texts from Greece, Egypt, Rome, China, and India.

The written evidence of herbal use for the treatment of diseases dates back to over 5000 years, to the Sumerians, who created lists of plants. Very sophisticated ships loaded with earthenware amphorae were built solely for the olive oil trade. In fact, olive oil trade may have been the source of wealth for this advanced Minoan civilization. The earliest reference to opium growth and use is in 3400 BC when the opium poppy was cultivated in lower Mesopotamia (Southwest Asia). The Sumerians referred to it as Hul Gil, the "joy plant." The Chinese book on roots and grasses "Pen T'Sao," written by the Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even today. The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 proscriptions referring to 700 plant species and drugs used for therapy such as pomegranate etc. Pharaonic Egypt used cumin as a medicine around 1550 BC. Saffron was used as medicine on the Aegean island of Thera.

In Homer's epics the *Iliad* and the *Odyssey*, created circa 800 BC, 63 plant species from the Minoan, Mycenaean, and Egyptian Assyrian pharmacotherapy were referred to. Some of them were given the names after mythological characters from these epics; for instance, Elecampane (Inula helenium L. Asteraceae) was named in honor of Elena, who was the center of the Trojan War. As regards the plants from the genus Artemisia, which were believed to restore strength and protect health, their name was derived from the Greek word artemis, meaning "healthy." As a digestive aid, Confucius wrote as far back as 500 BC of never being without ginger when he ate. It was around 500 BC that turmeric emerged as an important part of Ayurvedic medicine.

The works of Hippocrates (460–370 BC) contain 300 medicinal plants classified by physiological action. Wormwood and common centaury were applied against fever; garlic against intestine parasites; opium, henbane, deadly nightshade, and mandrake were used as narcotics; fragrant hellebore and haselwort as emetics; sea onion, celery, parsley, asparagus, and garlic as diuretics; and oak and pomegranate as astringents. Theophrast (371–287 BC) founded botanical science with his books *De Causis Plantarium*. In the books, he generated a classification of more than 500 medicinal plants known at the time. Pliny the Elder (23–79 AD) wrote about approximately 1000 medicinal plants in his book *Historia naturalis*. Pliny's works incorporated all knowledge of medicinal plants at the time.

In 65 AD, Dioscorides wrote his *Materia Medica*, a practical text dealing with the medicinal use of more than 600 plants. Charles the Great (742–814 AD), the founder of the reputed medical school in Salerno, in his "Capitularies" ordered which medicinal plants were to be grown on the state-owned lands. Around 100 different plants were quoted, which have been used to date such as sage, sea onion, iris, mint, common centaury, poppy, and marshmallow. Al-Kindi (800–870) contributed to the history of medicine. This scholar was heavily influenced by the work of Galen and also made unique contributions of his own to the field. In his Aqrabadhin (Medical Formulary), he described many preparations drawn from plant, animal, and mineral sources. "Not only is every sensation attended by a corresponding change localized in the sense-organ, which demands a certain time, but also, between the stimulation of the organ and consciousness of the perception an interval of time must elapse, corresponding to the transmission of stimulus for some distance along the nerves."

Ibn Sina, also known as Avicenna (980–1037 AD), combined the herbal traditions of Dioscorides and Galen with the ancient practices of his own people. His book spread through Europe during the eleventh and twelfth centuries. *Canon Medicinae* and *Liber Magnae Collectionis Simplicum Alimentorum Et Medicamentorum* by Ibn Baitar (1197–1248) included descriptions on 1000 medicinal plants. Vasco da Gama's journeys to India (1498) resulted in many medicinal plants being brought into Europe. Botanical gardens emerged all over Europe, and attempts were made for cultivation of domestic medicinal plants and of the ones imported from the old and the new world. Paracelsus (1493–1541) was one of the proponents of chemically prepared drugs out of raw plants and mineral substances.

The great pharmacologist of the Ming dynasty, Li Shizhen (b1518–d1593 AD), spent 30 years consulting some 800 texts and personally harvesting herbs for use in treatment to write the great classic, *Materia Medica*, containing 52 articles. Withering gives clinical details of how to prescribe extract of foxglove, or digitalis, in the treatment of dropsy and hints that it may be of use for heart disease.

In 1858, Louis Pasteur wrote that garlic killed bacteria. As he maintained, it was effective even against some bacteria resistant to other factors. He also noted that garlic killed *Helicobacter pylori*. Cocaine was first isolated (extracted from coca leaves) in 1859 by the German chemist Albert Nieman. In 1886, the popularity of the drug got a further boost when John Pemberton included coca leaves as an ingredient in his new soft drink, Coca-Cola. From 1966 to 1976, traditional doctors were purged from the schools, hospitals, and clinics, and many of the old practitioners were jailed or killed.

According to WHO, nearly 80% of the population rely on plants for their primary health care globally. Approximately 30,000–70,000 plant taxa are used as medicaments. This means that nearly 14–28% of the 250,000 identified plant taxa in the world and 35–70% of all species are used on our earth; more than 50 major drugs have originated from tropical plants.

The great surge of public interest in the use of plants for medical purposes has been based on the assumption that these resources will be available on a continuing basis. Among the medicinal plants there are many pharmacopoeial ones. From about 250,000 species of higher plants around the world, only 17% have been scholarly investigated for medical potential. The chemical and biological diversity of plants represents a potentially limitless renewable source for the use in the development of new pharmaceuticals. Traditional Chinese medicine used 5000 of them, whereas the Native Americans have used only 2564 herbs as medicine. The botanical wisdom accumulated by indigenous people has led to the establishment of the traditional systems of medicine. The pharmacologist Farnsworth Norman says that 89 plant-derived drugs currently prescribed in the industrial world have been found with the help of ethnobotanical approach.

Definite signs of plant cultivation first appeared in early Neolithic villages in the Near East around 7500–7000 BC. The initiation of food production in what could be called the “nuclear area” was based on the domestication of about 8 species of local grain plants. Olives were probably first brought into cultivation in the Levant. Many condiment and dye plants have been cultivated here for thousands of years, including coriander, cumin, saffron, and safflower. The core of first domestication of the above-mentioned plants and several others is mainly represented by what is called the “Fertile Crescent,” considered to be the cradle of civilization and covering the valleys of Tigris and Euphrates Rivers, the southern slopes of the Taurus Mountains, and the eastern shores of the Mediterranean Sea. Herodotus described the amazing fertility of the irrigated plains around this area.

The knowledge of medicinal plants spread widely in this region; even its conservation was achieved by the scholars from different faiths. Numerous treatises, in various languages, were written on the use of medicinal plants. The monumental and celebrated *Materia Medica* about herbal medicine and related medicinal substances was widely read for more than 1500 years. It was written in 78 AD by Pedanius Dioscorides, a physician, pharmacologist, and a botanist, who was born in Anazarba, today’s Tarsus in Turkey. Many plants out of the 950 drugs given by him grow wild in the area. The origin, morphological and pharmaceutical features of these plants were given, in addition to the illustrations. Many herbalists wrote numerous treatises. Abul-Abbas Ahmed, lbn ara Rumiya (d. 1239 AD) journeyed in North Africa, Syria, and Mesopotamia and described many plants in his book *The Botanical Journey*. One of the most original botanists of the thirteenth century was Rashid ad-Din ibn as-Suri, who lived in Syria between 1177 and 1243 AD, traveled in the Near East accompanied by a painter, described many unknown plants, and had them painted as fresh plants and drugs. Ibn al Baitar (1248 AD; Damascus) wrote a monumental book *Al Garni* (Collection on Remedies), which is a very valuable book about medicinal plants. One of the most well-known treatises on medicinal plants is *Dhakhirat Uli al-Albab* (Memorandum of Intelligent People) written by Dawud Al-Antaki who was born in Antakya. It contains an alphabetical annotated list of herbal drugs and medical terms. In Iraq, many herbalists wrote about the medicinal plants and their uses and many treatises appeared.

The effectiveness of foxglove from traditional herbal medicine in the eighteenth century has been helpful in the treatment of dropsy. More than 30 cardiac glycosides have been isolated from dried foxglove leaves including digitoxin and digoxin. All these are useful because they increase the force of heart contractions. Nearly 1500 kg of digoxin and 200 kg of digitoxin are prescribed to heart patients globally. The snakeroot plant was traditionally used for the treatment of insomnia in India. In 1949, German chemists extracted alkaloid reserpine from its roots, which is used today for the treatment of high blood pressure. Similarly artemisinin is the biologically active compound used today to fight malaria. It is a sesquiterpene lactone from wormwood and was first isolated in 1972 by Chinese chemists. Quinine is another example coming from *Chinchona* species used to treat malaria. Madagascar periwinkle is used today in the chemotherapy of childhood leukemia and for the treatment of Hodgkin's disease. The compound taxol with anticancer action comes from the bark of Pacific yew tree. The extract from opium poppy has been used since the time of Pharoes as pain killer. It contains morphine, codeine, and heroin alkaloids. The anesthetic drug cocaine too has been widely used as a local pain killer.

Understanding the relationship among medicinal plants used in traditional medicine systems can help identify plant materials with potential constituents applicable to modern medicine. Licorice has been used for the treatment of bronchial asthma in traditional medicine. Illiterate traditional healers living in the forests around the globe have used the herbs correctly for medicinal purposes. They learnt all about these through trial and error; there was some spiritual learning by ritual use of medicinal plants in religious ceremonies, like "invoking hidden power of the plants" and meditation; they followed by observing how apes and other animals use the plants; and finally they strived hard to preserve the oral tradition by passing their knowledge from generation to generation.

Investigation of plants used in traditional medicine to determine biological activities is a complicated process. It requires obtaining reliable ethnobotanical data on use in the traditional system, collecting specimens from the correct genera and species, investigating the activity of crude extracts and active principles, and analyzing the chemical structure, synthesis, and structural modification.

Nearly seven billion people and the plants live together on this planet. We need not forget that plants lived there for millions of years before us. A major difference is that plants can live without people, but people cannot live without plants. For a successful research on potential new sources of medicines from plants, each medicinal plant has hundreds of biologically active chemical compounds that work synergistically together as a result of natural selection. Each herb affects humans directly and indirectly. The former is based on the pharmacological action of its biologically active compounds, whereas the latter is related to interaction with other plants or drugs taken. Search for medicinal plants to cure epidemic diseases should include the plants from the geographical place, where these diseases originated and spread around. If a plant from a genus has significant medical value, it is not necessary that other plants from the same genus may have the same medical value—only difference is potency; other plants from the genus may have more or less potency. The geographical position, habitat, and correct identification of medicinal plants are very important. An identification of chemical compounds and genetic markers alone is not enough; we have to learn about their chemotaxonomy, molecular biology complemented by classical botanical methods. If a particular ethnic group has used plants for several generations continuously from one traditional herbal medicine system, those plants remain the first choice for treatment.

As many people globally depend on medicinal plants for health, their sustainability and conservation must be our first priority. We should do everything possible to preserve the plants for our future generations.

Izmir, Turkey; Amann, Jordan — Munir Ozturk
Jeddah, Saudi Arabia — Khalid Rehman Hakeem

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Contents

Molecular Biodiversity Convergence with Biogeography and Ethnobotany of Rare and Endangered Medicinal Plants from Northern Vietnam 1
N. V. Huy, H. V. Hung, R. T. Buckney, and L. F. De Filippis

Health and Illness as a State of Being Human 53
Nevin Turgay, Özlem Yılmaz, and Fehmi Akçiçek

Ethnobotanical Explorations in Telangana, the Youngest State in Union of India: A Synoptic Account 65
Sateesh Suthari, S. Raju Vatsavaya, and Narasimha Vara Prasad Majeti

Ethnobotany and Pharmacological Uses of *Elaeocarpus floribundus* Blume (Elaeocarpaceae) 125
Mohamad Fawzi Mahomoodally and Veedooshee Sookhy

Medicinal Plants Against Cancer 139
Sameen Ruqia Imadi, Isra Mahmood, and Alvina Gul

Herbals in Iğdır (Turkey), Nakhchivan (Azerbaijan), and Tabriz (Iran) 197
Munir Ozturk, Volkan Altay, Ernaz Altundağ, S. Jamshid Ibadullayeva, Behnaz Aslanipour, and Tuba Mert Gönenç

The Utilization and Conservation of Plants of Medicinal Value by Local Traditional Medicinal Practitioners and the Associated Indigenous Knowledge in Dawuro Zone of Ethiopia: Northeast Africa—An Ethnobotanical Approach 267
Moin Ahmad Khan, Mathewos Agize, Abraham Shonga, and Asfaw Tora

Medicinal Bryophytes Distributed in Turkey 323
Munir Ozturk, İsa Gökler, and Volkan Altay

A Comparative Analysis of the Medicinal Pteridophytes in Turkey, Pakistan, and Malaysia 349
Munir Ozturk, Volkan Altay, Abdul Latıff, Tabinda Salman, and Iqbal Choudhry

Medicinal Shrubs and Trees from the Nara Desert, Pakistan 391
Rahmatullah Qureshi

A Comparative Analysis of the Medicinal Plants Used for Diabetes Mellitus in the Traditional Medicine in Turkey, Pakistan, and Malaysia 409
Munir Ozturk, Volkan Altay, Abdul Latiff, M. Asad Ziaee, M. Iqbal Choudhry, Farzana Shaheen, and Cenk Durmuşkahya

Antidiabetic Plants of Pakistan 463
Mubashrah Munir and Rahmatullah Qureshi

Ethno-ecology, Human Health and Plants of the Thandiani Sub Forest Division, Abbottabad, KP, Pakistan 547
Waqas Khan, Shujaul Mulk Khan, and Habib Ahmad

Ethnobotanical Uses of Some Plants of Families Apocynaceae and Asclepiadaceae from the Northwestern Region of Ahmednagar District, Maharashtra 569
Mohan B. Waman and Mahendra S. Khyade

Plants, Food, and Health: Some Untold Truths 583
Ægir B. Kristiansson

Potential Medicinal Plants Used in the Hypertension in Turkey, Pakistan, and Malaysia 595
Munir Ozturk, Volkan Altay, Abdul Latiff, Samreen Shareef, Farzana Shaheen, and M. Iqbal Choudhry

***Epimedium elatum* (Morr & Decne): A Therapeutic Medicinal Plant from Northwestern Himalayas of India** 619
Sajad Ahmad Lone, Ajai Prakash Gupta, Malik Muzafar Manzoor, Pooja Goyal, Qazi Pervaiz Hassan, and Suphla Gupta

Indian Herbal Drug Industry: Challenges and Future Prospects 657
Musadiq H. Bhat, Ashok K. Jain, and Mufida Fayaz

Ethno-ecology of the Healing Forests of Sarban Hills, Abbottabad, Pakistan: An Economic and Medicinal Appraisal 675
Farhana Ijaz, Inayat Ur Rahman, Zafar Iqbal, Jane Alam, Niaz Ali, and Shujaul Mulk Khan

Exploring the Therapeutic Characteristics of Plant Species in the Chichawatni Irrigated Plantation Pakistan 707
Muhammad Arif, Waseem Razzaq Khan, Muhammad Khurram Shahzad, Amna Hussain, and Cao Yukun

Therapeutic Characteristics of Murree Plants: An Emerging Feature . . . 719
Waseem Razzaq Khan, Muhammad Arif, Sadaf Shakoor, M. Nazre, and M. Muslim

Plant Resources and Human Ecology of Tarnawai area, District Abbottabad, Pakistan . . . 731
Raheela Taj, Inayat Ur Rahman, Abbas Hussain Shah, Shujaul Mulk Khan, Aftab Afzal, Niaz Ali, Zafar Iqbal, and Farhana Ijaz

Licorice in Middle Asia . . . 757
F. O. Khassanov

Ethnobotany of Medicinal Plants for Livelihood and Community Health in Deserts of Sindh-Pakistan . . . 767
Ghulam Yaseen, Mushtaq Ahmad, Daniel Potter, Muhammad Zafar, Shazia Sultana, and Sehrosh Mir

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Index . . . 793

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Molecular Biodiversity Convergence with Biogeography and Ethnobotany of Rare and Endangered Medicinal Plants from Northern Vietnam

N. V. Huy, H. V. Hung, R. T. Buckney, and L. F. De Filippis

Introduction

Biogeography, Ethnobotany and Phylogeny

Plants fulfil the basic needs of humans with materials for existence, which can be medicinal, economic, food and fodder values. From any region plants can be lost, and the knowledge enclosed within them is also destroyed; sometimes it can disappear forever. In more recent times, plant conservation can be an emotive and a 'hot' issue; however, it is known that primarily due to overharvesting medicinal and some important food, plant populations have become severely reduced. Overexploitation of plant resources combined with improper harvesting and postharvesting techniques and lack of oversight and protection are increasing pressure on plant biodiversity (Ellegren 2008; Garnatje et al. 2017).

'Let food be thy medicine and medicine be thy food'; a statement attributed to Hippocrates (460–about 370 BC), which reflects the approach of the Greek physician to medicine and food, emphasizing for the first time the importance of diet and living habits in preventing illness and disease. In the past, a large group of plant species were used for the preparation of medicines and were also consumed as foods. This concept was well-established among people who traditionally gathered wild food plants, and the people were also aware of their health-beneficial properties. Nowadays, wild food plants are generally known to have high nutritional values, higher fibre and polyphenol contents and greater antioxidant capacity than the corresponding culti-

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vated species. Moreover, many wild green plants have been demonstrated to be effective in preventing chronic diseases, such as cardiovascular diseases and diabetes. Much of this knowledge has been orally passed from generation to generation which has led to the development of the traditional health-care system, practised in various countries of the world (Savo et al. 2015; Qureshi et al. 2016).

There are over 300,000 species of seed plants around the globe, of which about 60% of plants have found medicinal and food use in post-Neolithic human history. Nowadays, people collect plants for medicine and food not only from the wild but also through artificial cultivation, which are an indispensable part of human civilization. Medicinal plants are also essential raw materials of many chemical drugs, e.g. the blockbuster drugs for antimalaria and anticancer therapy, and, currently, more than one-third of clinical drugs are derived from botanical extracts and/or their ingredients (Henry 2012; Shaheen et al. 2017). Different vegetation associations classified through biogeography and ethnobotany of local ecological knowledge are called ecotopes (Table 1). The term may also be used to describe how people view, use and occupy their land. Forests around the world in a large part have been transformed into ‘cultural ecotypes’, since many forests are influenced by natural disturbances, as well as by human disturbances. The vegetation patterns, which result from disturbances, reflect complex interactions between biotic and abiotic characteristics (Kellogg et al. 2016), as well as cultural characteristics (Chivenge et al. 2015; Hao and Xiao 2015).

One selection criterion for plant characteristics used by biogeographers and ethnobotanists is based on phylogeny (Table 1). A non-random distribution of used medicinal plant species across families has been observed in several parts of the world (e.g. Medeiros et al. 2013). Plants within the same family, with close evolution ties, are more likely share similar secondary compounds which may have similar or equal medicinal properties (e.g. Yessoufou et al. 2015), and this has been intuitively discovered by many traditional communities. Furthermore, plants that are evolutionarily closely related have generally more total uses than those that are evolutionarily isolated. Promising predictions of medicinal plant uses have been developed based on the conjunction of ethnobotanical, phytochemical and molecular phylogenetic data (Massana 2015). The use of the same (or closely related) species in the same way in different cultures indicates that different and often noninteracting human groups have independently acquired this knowledge. This results from the fact that some plants have similar morphological characteristics because they have a close phylogenetic placement (Leonti 2011).

Convergence of Ethnobotany and Molecular Biology

Ethnobotanical studies discover plant resources that can be used for targeting novel compounds leading to the development of new medicaments for treating complicated and minor diseases. Today, ethnobotany and ethnopharmacology (Table 1) are being used for targeting new compounds. Tropical regions are rich in plant diversity and may play key roles in providing germplasm with new bioactive compounds (Hedrick 2004; Garrick et al. 2015). Plants and humans are engaged in a dynamic

Table 1 Definitions, terms and features commonly used in this review related to biogeography, ethnobotany, phylogeny, population statistics and population genetics

Term or feature	Definition
ANCOVA	Analysis of covariance
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
Biodiversity	The number, variety and genetic variation of different organisms found within a specified geographic region. A term that describes the number of different species that live within a particular ecosystem
Biogeography	The study of the geographical distribution of living things. A biogeographic region is a large, generally continuous division of the Earth's surface having a distinctive biotic community
Bioprospecting	The search for plant species from which medicinal drugs and other commercially valuable biocompounds can be obtained. The process of discovery and commercialization of new products based on biological resources, only recently begun to incorporate indigenous knowledge
Cluster analysis	Clustering is the task of grouping a set of objects in such a way that objects in the same group (called a cluster) are more similar (in some sense) to each other than to those in other groups (clusters) used in many fields
Ecotypes	A group of organisms within a species that is adapted to particular environmental conditions and therefore exhibits behavioural, structural or physiological differences from other members of the species
Genomics	An interdisciplinary field of science focusing on the structure, function, evolution, mapping and editing of genomes or an organism's complete set of DNA, including all of its genes, DNA sequencing and analysis
MDS	Multidimensional scaling
PCA	Principle component analysis
Pharmacology	The branch of biology concerned with the study of drug action, where a drug can be broadly defined as any man-made, natural or endogenous (from within body) molecule which exerts a biochemical or physiological effect on the cell, tissue, organ or organism. More specifically, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function
Phylogeny	The sequence of events involved in the evolution of a species and genus. The evolutionary development and history of a species or higher taxonomic grouping of organisms, through evaluation of heritable traits, such as DNA sequences or morphology under a model of evolution of these traits
Phytochemical	The study of chemicals derived from plants and strives to describe the structures of the large number of secondary metabolic compounds found in plants, the functions of these compounds in human biology, their biosynthesis and in many cases the health benefits of these compounds
Pleiotropy	When one gene influences two or more seemingly unrelated phenotypic traits and the single gene is capable of controlling or influencing multiple (and possibly unrelated) phenotypic traits
Population genetics	A subfield of genetics that deals with genetic differences within and between populations, due to adaptation, speciation, inheritance and population structure; a part of evolutionary biology usually using statistical analysis

Definitions extracted and modified from the authors relationship, where plants evolve creating biodiversity and humans develop strategies and solutions to use them. In this relationship, plants evolve secondary metabolites to protect themselves from being used excessively, and people find ways to use these metabolites to their advantage.

Thus, we propose to use the term ‘convergence’, to label similar uses for plants included in any node of a phylogeny relationship. Determining the phylogenetic and genetic relationships among plant species could be an appropriate tool for discovering new drugs based on recorded plant medicinal uses and analysis of ethnobotanical data. New perspectives have emerged with the development of new molecular tools, especially for DNA sequencing; and these enable phylogenetic reconstruction and clustering of potentially useful plants (Robertson and Richards 2015; Maestri 2017). For example, extracts from *Pterocarpus* Jacq. spp. (a phylogenetic cluster) have the same medicinal uses in geographically distant areas, namely, the neotropics, tropical Africa and Indomalaya. This example of ethnobotanical convergence illustrates that different cultures have discovered related plants that are used to treat similar disorders. Another example of ethnobotanical convergence is provided by the spices used as condiments for two products in different geographical and cultural areas. Pizza in Western cultures is seasoned with *Origanum vulgare* L., and near Eastern similar food (manousheh) is prepared using another species of the same genus as a condiment, *O. syriacum* L. Both taxa are phylogenetically very close, implying a similar chemical composition and thus a similar use. In addition to the phylogenetic approach, the large data sets obtained using ‘molecular biology’ techniques (e.g. genomics, transcriptomics, proteomics and metabolomics; Table 1) and their analyses using bioinformatic tools are more often used for identifying plants with popular ethnobotanical uses and the most promising taxa (or genes within those taxa) for medicinal and culinary use (Keatinge et al. 2011; Kahane et al. 2013). These molecular methods and the resulting data sets also provide a better understanding of the evolutionary history of medicinal and food plants and are further developments in ethnobotanical convergence with molecular biology (He et al. 2017).

Genetic Diversity and Population Genetics

Biodiversity is the material foundation of human survival and development and also is an important symbol to measure the environmental quality status and degree of ecological state in a region or a country. Biodiversity refers to the sum total of different animals, plants and organisms living on Earth and may include species and genetic diversity, as well as the variety of habitats and ecosystems where they live (Table 1). Biodiversity functions to provide direct and beneficial products to humans, regulation of climate and the environment, formation of unique cultures and other important functions (Holliday et al. 2017).

Tropical and sub-tropical forests cover only about 7% of the Earth’s land yet contain up to 50% of all plant species. These regions are important areas of biodiversity, containing many endemic vascular plants, yet the nature and integrity of these important ecological zones are being impacted on at a greater rate than ever. Therefore much of the biodiversity in these areas is unlikely to survive without effective protection. This high diversity is in part due to steep ecological gradients, including microclimatic conditions, sharply defined ecotones and a lower amount of anthropogenic disturbance compared to temperate and dry forests. The distribution of plant species within tropical and sub-tropical national parks at present has been subjected to less human impacts and is likely to be less fragmented (Ford-Lloyd et al. 2011).

A good example is Vietnam where it is stated that 58% (19 million hectares) of total land is legally classified as forest, but ecologically speaking only part of this area actually possesses forest vegetation. Total forest cover has declined steadily throughout the twentieth century, and this decline has accelerated in recent decades. Only three million hectares are considered to possess well-stacked healthy forests; and old-growth forest is estimated to be only two million hectares. In the north and north-west regions of Vietnam, forest cover has been reduced from 95% in 1943 to between 14 and 24% in 1995 (Dang 2015). Conservation of these remaining forests is essential; however, priorities for conservation must use as estimators a number of economic and evolutionary criteria to be effective, and rare and endangered plants must form a solid basis for conservation strategies. Hence, it is important to establish sound criteria and a set of guidelines for the conservation of rare species and at the same time collect genetic diversity data to help formulate a sound management plan for endangered species (Comadran et al. 2012; MacDicken et al. 2016).

Natural Foods and Biopharmaceuticals

It is estimated that 80% of the world's population lives in developing countries and that over 80% of the world's population rely on plant-derived foods and medicines for their primary health care. Based on experience, people in the past knew therapeutic potential of medicinal plants without rationale of their efficacy. Because of advancement, we have a better understanding of the healing powers of plants due to the presence of multifunctional chemical entities for treating complicated health conditions. The plant kingdom is an implicit gold mine of new chemical compounds which are still waiting to be explored. It is estimated that there are approximately 500,000 to 750,000 species of higher plants existing on Earth and less than 10% of them have been examined for their biochemical constituents. The importance of ethnobotany must therefore be as an interdisciplinary science.

Traditional medicinal practice (TMP) encompasses a holistic worldview, which reflects that of the World Health Organizations definition of health, that is, one of 'physical, mental and social wellbeing and not merely the absence of disease or infirmity' (Leonti 2011; Savo et al. 2015). This worldview recognizes good health as a complex system involving interconnection with the land, recognition of spirit and ancestry and social, mental, physical and emotional wellbeing both of the individual and the community. Indigenous people view ill health as the result of one of three causes—a natural physical cause, a spirit causing harm and/or a sickness due to sorcery. Traditional healers (THs) are found in most societies and are often part of a local community, culture and tradition, and they continue to have high social standing in many places, exerting influence on local health practices (Hedrick et al. 2013).

Traditional healing is the oldest form of structured medicine and was originally an integral part of seminomadic and agricultural tribal societies. Archaeological evidence for its existence dates back to only around 6000 BC; but its origins probably date back to well before the end of the last Ice Age (Smith and Eyzaguirre 2007). There were and still are differences between the principles and philosophy of TMP, although there are also many fundamental similarities that arise from the profound knowledge of natural laws, and the understanding of how these influence living things, which are shared by all traditional healers. Major factors which affect medicinal plant diversity loss include razing for pastures, forest encroachment, soil erosion, over-collection, agro-system use, poverty, forest fires and invasive species intensifying in that environment. Climate variations and extremes may be additional influences on dispersal and richness of plant varieties. Deforestation, illegal trade, habitat loss, growing demand for natural products, industrial pollution and lack of adequate knowledge and training are other threats (Leonti 2011; Hao and Xiao 2015).

Aims and Scope

In this chapter we provide an overview of plant biogeography and ethnobotany and the multidisciplinary approach and convergence with molecular biology. We cover traditional phylogenetic approaches to drug discovery and move onto new methods (i.e. gel, fragment, size and sequence based) appropriate for use in molecular biology. We provide web-based resources available for use in plant molecular research, and we describe gel-based methods for use as molecular markers and use in population genetic diversity studies for rare, endangered and drug plants (Datta et al. 2010; Huang et al. 2012; Salgotra et al. 2014; Williams et al. 2014; Unamba et al. 2015). We also describe the current status of resources and technologies for transcriptomics, proteomics and metabolomics; however, some of these fields are more comprehensively described in other literature (Ekblom and Galindo 2011; Alvarez et al. 2012; Egan et al. 2012; Varshney et al. 2014; Guttikonda et al. 2016). Species of plants used in the research described in this review have been divided into sections based on rare and endangered plants and plants used for drugs and foods. Resources and techniques for use in next-generation sequencing (NGS) research will be discussed, and the integration of computer programmes and bioinformatics across plants in comparative genomics is outlined (Llaca 2012; Ray and Satya 2014; Barabaschi et al. 2016; Thottathil et al. 2016; De Filippis 2018). Currently, traditional and deep sequencing users are faced with an abundance of marker and sequencing data and analysis tools, both publicly and commercially available. We intend to point out various aspects to be considered when choosing an analytical tool and emphasize the relevant challenges and possible limitations so as to assist the user in picking the most suitable platforms and methods.

The four plant species under detailed investigation in this study have their highest frequency in the north of Vietnam adjacent to Ba Be and Cat Ba National Parks (Fig. 1), and all four are represented by small fragmented populations with a high risk of local extinction. *Sinocalamus mucclure* (string bamboo) and *Markhamia stipulata* are currently restricted to the volcanic limestone ridges of Ba Be National Park, and only string bamboo has apparently been recorded elsewhere, in a localized region of Southern China. *Cycas fugax* has only been described in Phu Tho Province, 200 m above sea level in very low numbers and in few locations. *Celastrus hindsii* is more widely distributed up to an altitude of 2500 m in regions of Northern Vietnam, China, India and Myanmar. Unfortunately for all four species, their preferred habitat has also been favoured by local tribal people for collection in traditional medicine (Ban 2003; Hung et al. 2011). Land clearance and overuse of these four species have led directly to the endangerment of populations, through direct removal of individuals, and the division of previously continuous populations into smaller and smaller fragments separated by inhospitable terrain (Dinh and Bui 2010; Dang 2015; Shaheen et al. 2017).

Traditional Ethnobotanical Methods

Natural and Social Sciences: Crucial Resources for Humans

Biogeography seeks to understand the underlying biotic and abiotic processes responsible for the spatial and temporal distributions of organisms (Tables 1 and 2). Evolutionary biogeography uses phylogenetic data to integrate concepts from phylogenetic ecology and evolutionary biology (Weckerle et al. 2011) with ecological and historical biogeography (e.g. environmental filters, dispersion, variance statistics); the goal is to elucidate biogeographic patterns and processes in a historical and evolutionary context. Biogeographical studies are traditionally focused on species diversity and distribution. However, in recent decades, the study of traits across spatial and temporal scales has proved useful for explaining and describing the diversity of forms on a biogeographical scale, thus creating the discipline of functional biogeography and 'the analysis of the patterns, causes, and consequences of the geographical distribution of the diversity of form and function' has lead to a convergence of natural and social sciences (Tables 1 and 2).

Ethnobotany is also located at a critical interface of natural and social sciences and has numerous applications to plants, especially related to human health and wellbeing (Table 1). Plants with exceptional traits and market or end-user potential must be identified; genetics and breeding can then help to resolve some of the current issues which are preventing the more extensive use of these species, but it must

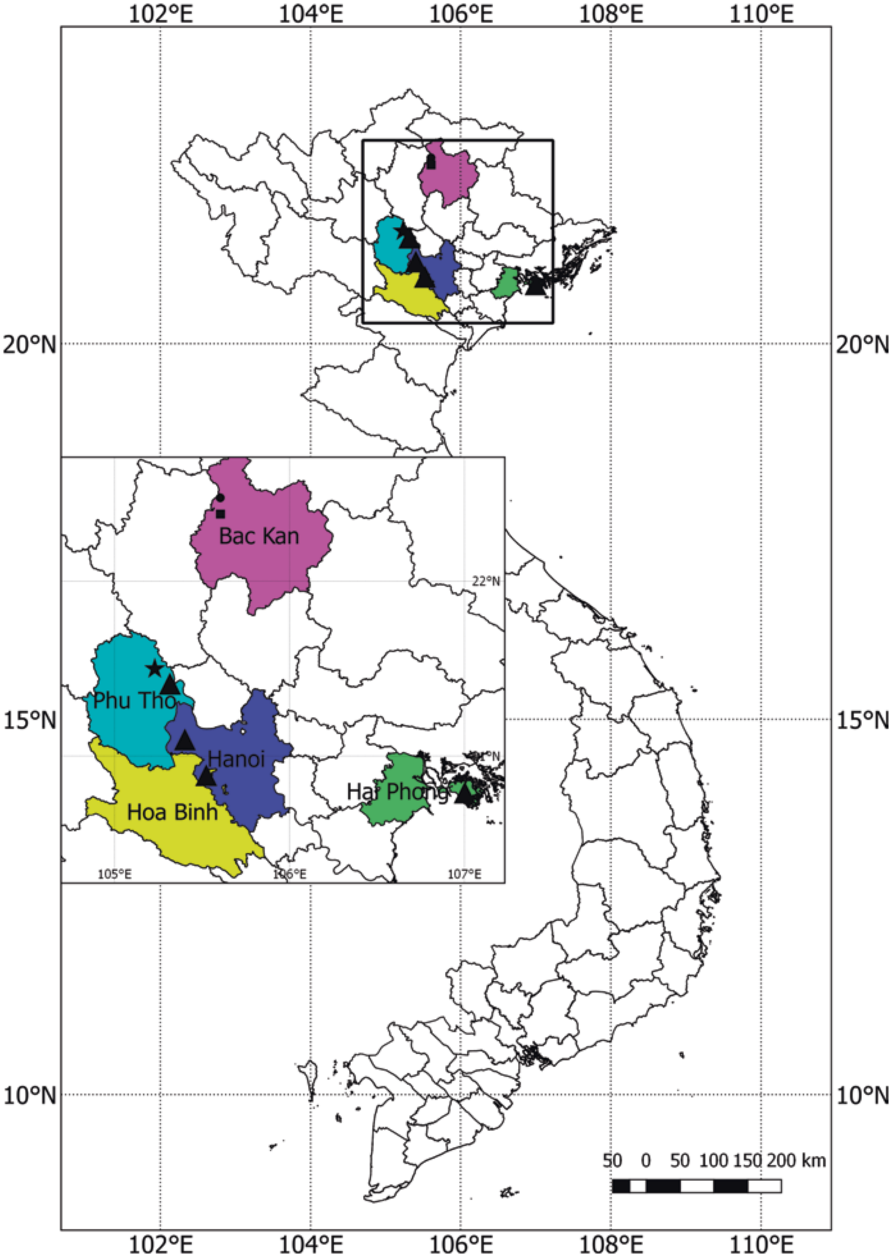

Fig. 1 Map of Vietnam including the locations of populations and sampling sites for the two rare and endangered plants and the two drug and food plants used in this study

Table 2 Definitions, terms and features commonly used in this review related to molecular biology, bioinformatics and next-generation sequencing (NGS)

Term or feature	Definition
AFLP	Amplified fragment length polymorphism
Bioinformatics	An interdisciplinary field that develops methods and software tools for understanding biological data. An interdisciplinary field of science, combining computer science, biology and mathematics and referencing to specific analysis 'pipelines' that are repeatedly used in the field of genomics
DNA barcodes	A taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. It differs from molecular phylogeny in that the main goal is not to determine patterns of relationship but to identify an unknown sample in terms of a preexisting classification. The most commonly used barcode region is the internal transcribed spacer between rRNA genes, and in plants multiple regions are now advocated.
EST	Expressed sequence tags
Ethnobotany	The study of a region's plants and their practical uses through traditional knowledge of a local culture and people. The practical uses of local flora for many aspects of life, such as plants as medicines, foods and clothing
Genetic diversity	The total number of genetic characteristics in the genetic make-up of a species and serves as a way for populations to adapt to changing environments. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary
ESI-MS/MS	Electrospray ionization tandem mass spectrometry (MS)
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
Proteomics	The large-scale study of proteins, a vital parts of living organisms, with many functions. Proteomics is an interdisciplinary domain, and the proteome is the entire set of proteins that are produced or modified by an organism or system
RADseq	Restriction site-associated DNA sequencing
RAMP	Random amplification microsatellite polymorphism
RAPD	Random amplification polymorphic DNA
RNAseq	RNA sequencing
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SNP	Single-nucleotide polymorphism
SSR	Simple sequence repeats
TE	Transposable elements

Definitions extracted and modified from the authors

be a focused effort and is likely to be enabling, rather than transformative. For instance, with regard to geographical distribution, a species that might be underutilized in some regions may not be in other areas (Foley et al. 2011). Metacommunity analysis suggests that the influence of environmental factors on mean trait variation relies heavily on spatial biogeographical clade sorting. This implies that biogeographical lineage distribution should be taken into account in analyses seeking to correlate environmental variables with mean trait variations.

Ethnobotany Information: Bioprospecting and Traditional Uses

During evolution, plants develop tactics of chemical defences, leading to the evolution of specialized metabolites with diverse potencies. A correlation between phylogeny and biosynthetic pathways could offer a predictive approach, enabling more efficient selection of alternative and/or complementary plants for guaranteeing clinical use and novel food discovery. This relationship has been rigorously tested and the potential predictive power subsequently validated. A phylogenetic hypothesis was put forwards for medicinal plants in the subfamily Amaryllidoideae (Amaryllidaceae) based on parsimony and tested whether alkaloid diversity and activity in bioassays related to the central nervous system were significantly correlated with molecular phylogeny. Evidence for a significant phylogenetic signal in these traits has been found, but the effect was not that strong (Fierst 2011; Archmiller et al. 2015).

Bioprospecting for new drugs with a botanical origin and for new food crops has traditionally been based on ethnobotanical information. Ethnobotanically directed bioprospecting has become more powerful than random assays for finding and identifying bioactive compounds from plants. Aspirin (from *Filipendula ulmaria* L. Maxim), codeine and papaverine (from *Papaver somniferum* L.), colchicine (from *Colchicum autumnale* L.), digoxin and digitoxin (from *Digitalis purpurea* L.), tetrahydrocannabinol and cannabidiol (from *Cannabis sativa* L.) and vinblastine and vincristine (from *Catharanthus roseus* L. Don) are among the most important classical drugs developed from ethnobotanical leads (Leonti 2011; Ahmad et al. 2012). The first evidence for the anticancer properties of paclitaxel, from *Taxus* L. spp., came from its toxic effects on murine leukaemia cells, in agreement with the well-known general toxicity of these genera of plants. The success of *Taxus*-related anticancer products highlights the promising role of plant products in drug development. More recently, during the avian flu epidemic, oseltamivir was developed from *Illicium verum* Hook based on ethnobotanical data from Chinese traditional medicine. Ethnobotanical records have also led to the isolation and development of artemisinin (from *Artemisia annua* L.) as a powerful antimalarial drug (Yessoufou et al. 2015), whose relevance was recognized with the 2015 Nobel Prize in Physiology or Medicine.

Examples from a social point of view of high nutritional foods are leafy vegetables, a group of several species used by millions of people in South America and sub-Saharan Africa. Yet poor marketing makes them largely underutilized in economic terms, and any underutilized plant species can make an important contribution to a better diet for local communities (Weinberger 2007; Yang and Keding 2009). Oca (*Oxalis tuberosa*), ulluco (*Ullucus tuberosus*) and mashua (*Tropaeolum tuberosum*), three traditional Andean tuber plants, are richer in vitamin A and vitamin C than the well-known potato. Quinoa (*Chenopodium quinoa*), cañahua (*Chenopodium pallidicaule*) and amaranth (*Amaranthus caudatus*), grains from the Andean region, contain far higher amounts of certain essential amino acids than wheat. The leaves of black nightshade (*Solanum nigrum*) can provide significant amounts of calcium, iron, phosphorous, vitamin A, vitamin C, proteins and the amino acid methionine, scarce in commonly marketed vegetables (Uusiku et al. 2010; Kahane et al. 2013).

Phylogeny and Chemical Methods

Medicinal plants synthesize an arsenal of protective (even toxic) molecules, most of which are secondary metabolites, which can be ingested by animals and humans. Plants evolving in the same lineage have more medicinal uses than evolutionarily isolated species, and the diversity of medicinal uses is correlated with the evolutionary history of the species. Species-rich clades are more likely than species-poor clades to contain taxa with more uses, while ancient taxa are less abundant in the flora and, therefore, are less used in traditional medicine (Savolainen et al. 2013; Rai et al. 2017). Given that chemical properties are evolutionarily conserved (Weckerle et al. 2011), bioscreening could be targeted to the lineages identified as 'hot nodes' for medicinal properties. Current nature-derived drugs come mostly from drug-productive families that tend to be clustered rather than scattered in phylogenetic trees. Only 62 of the 457 families of angiosperms and gymnosperms are used as sources for medicinal drugs (Xu et al. 2011; Memon 2012). As a result of evolution, species that have a wide geographical distribution may be more capable of synthesizing metabolites that enable them to adapt to such a wide distribution compared with species with a restricted distribution and with a local evolutionary history.

Nevertheless, the relationship between one specific bioactive compound and medicinal activity is not always clear, complicating the phylogenetic prediction of plant use. Phylogenomics can be integrated into the flowchart of drug discovery and development and extends the field of pharmacophylogeny at the molecular level. Phyloproteomics can also be used in a proteome-based phylogeny study and may be used to examine the evolutionary relationship at the epigenomic level, and phylometagenomics is also applicable in the exploration of medicinal plant-associated microbiota (Albert 2013; Hao and Xiao 2015).

Metabolomics and Proteomics: Evolutionary-Conserved Traits

The rapid development of the main techniques used in the analyses of metabolites (e.g. gas chromatography, high-performance liquid chromatography and nuclear magnetic resonance) is increasing the application of metabolomics in many aspects of natural drug (and food) discoveries (Zhang et al. 2010; Saxena and Cramer 2013; Jensen et al. 2016). Metabolomics, which is designed to provide general qualitative and quantitative profiles of metabolites in organisms exposed to different conditions, enables us to monitor the spatial and temporal distribution of target phytochemicals. In fact, assigning bioactive compounds from complex mixtures is a central challenge of natural product research. The combination of bioassay-guided fractionation with untargeted metabolite profiling improves the identification of active components (Wong et al. 2014). Metabolomics is also enabling a better understanding of medicinal plants and the identification of important metabolic quantitative trait loci for enhanced breeding. The integration of the metabolomics approach with genome-based functional characterizations of gene products for ethnobotanically important plants is helping to accelerate the discovery of novel biosynthetic pathways of specialized bioactive metabolites.

Biochemical integration has strongly enhanced the potential discovery and production of pharmaceutical and culinary products. For example, the production of the antimalarial drug artemisinin is being enhanced via traditional breeding, with new high-yielding hybrids to convert *A. annua* into a robust cropping system, and by the reconstitution of the biosynthetic artemisinin pathway in re-engineered microbial hosts (Ahmad et al. 2012). Genomics, proteomics and metabolomics are high-throughput technologies that may help speed up the determination of the mode of action of phytomedicines and allow investigation of herbal extracts without prominent active principles. Although metabolomics and proteomics techniques have generally proven valuable, they still face substantial challenges, including large-scale metabolite identification. However, further development of the metabolomics field in general could provide better tools for the discovery of the next generation of natural products inspired by popular knowledge gathered in ethnobotanical studies and enhanced by recent phylogenetic approaches.

Meta-analysis was most succinctly defined as 'the analysis of analyses'. In other words, the authors of a meta-analysis compile and quantitatively synthesize the results of available and pertinent studies using a meaningful common statistic to address a specific research question (Frankham 2015). Such meta-analytical techniques are recommended to synthesize the available literature because they often have higher statistical power than an individual primary study, due to the increased precision of the summary effects (Connor et al. 2011; Madden and Paul 2011). However, the power of meta-analysis relies on very specific methodological and statistical treatment of the individual studied. Meta-analysis can be applied to traditional biogeography studies but is better suited to studies using molecular methods and NGS approaches.

Molecular Ethnobotanical Methods

Molecular DNA Methods

For about 25–30 years, DNA markers have been the most widely used molecular markers in plants, owing to their abundance and polymorphism. Most of these markers can be selectively neutral because they are usually located in non-coding and non-regulatory regions of DNA (Hoang et al. 2009; Allendorf 2017). The first plant DNA markers were based on difficult restriction fragment length polymorphisms (RFLPs) and Southern blot-based methods. Eventually these were replaced