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Markets, mixtures and molecular methods

Investigating medicinal plant and edible orchid diversity in Tanzania and Zambia

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ACTA
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UPSALIENSIS
UPPSALA
2018

ISSN 1651-6214
ISBN 978-91-513-0376-5
urn:nbn:se:uu:diva-355182

Dissertation presented at Uppsala University to be publicly examined in Zootissalen, Villavägen 9, Uppsala, Thursday, 13 September 2018 at 09:00 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Prof. Julie Hawkins.

Abstract
Veldman, S. 2018. Markets, mixtures and molecular methods. Investigating medicinal plant and edible orchid diversity in Tanzania and Zambia. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1689. 36 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0376-5.

Medicinal plants are an important source of primary healthcare for many people in Tanzania. These medicinal plants are harvested from the wild, and increasing commercial trade poses a serious threat to local plant populations. Currently it is unknown which species are traded and in what amounts. Across the southwestern border in Zambia, the traditional dish *chikanda* has transformed from a niche product to being a mainstream delicacy. One of the main ingredients are wild-harvested orchids, and these have become depleted throughout the country as an effect of the increased trade. It is unclear which orchid species are targeted and might be at risk of overharvesting. The aims of my doctorate are to map harvest and trade of Tanzanian medicinal plants and Tanzanian and Zambian edible orchids, to investigate whether species that are traded on local markets can be identified using molecular methods such as DNA barcoding and metabarcoding and identify conservation issues arising from wild-harvesting of medicinal plants and edible orchids.

In **Paper I** DNA metabarcoding analysis of Tanzanian *chikanda* cake show the presence of 17 different orchids species belonging to the genera *Disa*, *Satyrium* and *Habenaria*, and in **Paper V** the analysis of *chikanda* tubers sold on Zambian markets reveals that at least 16 orchid species from 6 different orchid genera are targeted in local orchid trade. **Paper II** describes a quantitative market survey of the non-woody, non-powdered medicinal plants sold on Kariakoo market in Dar-es-Salaam that shows that a total of 67 species are traded in an annual volume of nearly 31 tonnes of fresh and dried medicinal leaves, seeds and fruits with an estimated value of 200,000 USD. For **Paper III** 873 medicinal plant products were analysed using DNA barcoding, literature and morphology to determine which species are traded on the Dar-es-Salaam and Tanga markets. In total, 509 identifications could be made corresponding to 91 species, 124 genera and 65 plant families, and several cases of over- and under-differentiation were detected. **Paper IV** builds upon the identifications in **Paper III** to determine in what amount the medicinal plant species present at the local markets are traded and to investigate if commercial trade poses a threat to local plant populations. It was found that several of the most highly favored medicinal plants were perceived to becoming more difficult to obtain in the wild.

This thesis shows that DNA barcoding is a powerful rapid identification method for morphologically unidentifiable specimens. It also shows that commercialization of wild-harvested plant products threatens local plant populations, and highlights the need for conservation measures to avoid local extinction of economically and socially important plant species.

Keywords: DNA barcoding, Africa, Species delimitation, Orchids, Medicinal plants

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ISSN 1651-6214
ISBN 978-91-513-0376-5
urn:nbn:se:uu:diva-355182 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-355182)

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Veldman, S.,** Gravendeel, B., Otieno, J.N., Lammers, Y., Duijm, E., Nieman, A., Bytebier, B., Ngugi, G., Marcos, F., van Andel, T.R., de Boer, H.J. (2017). High-throughput sequencing of African chikanda cake highlights conservation challenges in orchids. *Biodiversity and Conservation*, 1-18.

II Posthouwer, C., **Veldman, S.**, Abihudi, S., Otieno, J.N., van Andel, T.R., de Boer, H.J. (2018). Quantitative market survey of non-woody plants sold at Kariakoo Market in Dar-es-Salaam, Tanzania. *Journal of ethnopharmacology,* 222, 280-287.

III **Veldman, S.**, Ju, Y., Abihudi, S., van Andel, T.R., Otieno, J.N., de Boer, H.J. Good things come in threes – identification of medicinal plant species traded at Tanzanian markets using a combination of literature, molecular and morphological methods. (Manuscript).

IV **Veldman, S.**, Abihudi, S., Posthouwer, C., van Andel, T.R., Otieno, J.N., de Boer, H.J. Trade in Tanzania's threatened trees – a quantitative market survey of medicinal plants used in Dar-es-Salaam and Tanga. (Manuscript).

V **Veldman, S.**, Kim, S.-J., van Andel, T.R., Bello Font, M., Bone, R.E., Bytebier, B., Chuba, D., Gravendeel, B., Martos, F., Mpatwa, G., Ngugi, G., Vinya, R., Wightman, N., Yokoya, K., de Boer, H.J.Trade in Zambian edible orchids - molecular identification reveals the use of previously undocumented orchid taxa for *chikanda*. (Manuscript).

Published papers and popular scientific articles not included in this thesis.

I **Veldman, S**., Otieno, J.N., Gravendeel, B., van Andel, T.R., de Boer, H.J. (2014). Conservation of endangered wild harvested medicinal plants – Use of DNA barcoding. In: Novel Plant Bioresources: Applications in Food, Medicine and Cosmetics (Edited by A. Gurib-Fakim). Wiley & Sons, London.

II **Veldman, S**., Otieno, J.N., Gravendeel, B., van Andel, T.R., de Boer, H.J. (2014). Efforts urged to tackle thriving illegal orchid trade in Tanzania and Zambia for *chikanda* production. TRAFFIC Bulletin 10/2014; 26(2):47-50.

III **Veldman, S**., Otieno, J.N., Gravendeel, B., de Boer, H.J. (2014). Species assessment in African orchid cake. The Barcode Bulletin 07/2014; 5(2):10-11.

IV Otieno, J.N., Abihudi, S., **Veldman, S.**, Nahashon, M., Andel, T.R. van**,** de Boer, HJ. (2015). Vernacular dominance in folk taxonomy: A case study of ethnospecies in medicinal plant trade in Tanzania. *Journal of Ethnobiology and Ethnomedicine 11:10*.

V Hinsley, A., de Boer, H.J., Fay, M.F., Gale, S.W., Gardiner, L.M., Gunasekara, RS., Kumar, P., Masters, S., Metusala, D., Roberts, D.L., **Veldman, S**., Wong, S., Phelps, J. (2017). A review of the trade in orchids and its implications for conservation. *Botanical Journal of the Linnean Society*.

Contents

Abbreviations

BLAST	Basic Local Alignment Search Tool
CITES	Convention on International Trade in Endangered Species of Wild Flora and Fauna
COSTECH	Tanzania Commission for Science and Technology
DNA	Deoxyribonucleic acid
HDI	Human Development Index
HTS	High-throughput sequencing
ITM	Institute of Traditional Medicine
MUHAS	Muhimbili University of Health and Allied Sciences
NCBI	National Center for Biotechnology Information
NTFP	Non-timber forest product
PCR	Polymerase Chain Reaction
PIC	Prior Informed Consent
TANAPA	Tanzania National Park Authorities
TAWIRI	Tanzania Wildlife Research Institute
TCM	Traditional Chinese Medicine
TEK	Traditional Ecological Knowledge
TZS	Tanzanian Shilling
UNDP	United Nations Development Programme
WHO	World Health Organisation

Introduction

1.1 Ethnobotany

Throughout history plants have been used for a myriad of purposes. Plants provide people with food, medicine, timber, fibers and contribute to many types of ecosystem services. Ethnobotany essentially is the study that looks at the relationship between plants and people, although the definition and interpretation of the concept of ethnobotany has certainly changed over the years. The earliest article describing the importance of ethnobotany identifies four main purposes with ethnobotanical studies: 1) elucidating the cultural position of tribes who use the plants for food, shelter or clothing; 2) shedding light on the past distribution of plants; 3) determining ancient trade routes; and 4) suggesting new lines of manufacture at the present day (Harshberger, 1896). Nowadays the focus within ethnobotany has rather shifted towards a more applied form, where ethnobotany amongst others contributes to shedding light on the conservation status of plants, the sustainability of wild-plant harvest and to enhancing healthcare, food security and nutrition (Hamilton et al., 2003). Major advancements have been made concerning scientific and analytical methods applied within the field, including the of use of standardized quantitative methods, comprising a variety of indices and statistical methods making hypotheses testable and the research reproducible (Martin, 2004; Hoffman and Gallaher, 2007; van Andel et al., 2012; Weckerle et al., 2018). Moreover, the intellectual property rights of indigenous people have become increasingly recognized and international legislation has been put into place to assert these rights and to monitor the use of genetic resources by foreigners (UNEP, 1998).

1.2 Utilization of and threats to wild plant resources

Historically, plants and plant products have been gathered circumstantial or opportunistically as well as occasionally more extensively for accumulation by hunter-gatherer populations throughout the world (Zvelebil, 1994). However, since the transition to agriculture and sedentary lifestyles, more and more plants started to be cultivated around the house (Hillman & Davies, 1990; Diamond, 2002). Nevertheless, numerous plants are still harvested from the wild, i.e., to supplement subsistence income, because the plants are not easy to cultivate or take many years before they can be harvested, because the wild plant material is considered to be of higher quality, or just because they are available and easily accessible (Cunningham, 1993; Schippmann et al., 2002). Harvesting practices and local ecosystem management vary amongst different communities and are often influenced by mechanisms such as traditional ecological knowledge transmission, understanding of ecological complexity and social awareness (Berkes, 1993).

The extent to which plant resources are used are also a major factor influencing the impact of harvesting: i.e. whether wild-harvested plants are used on household scale or if they are commercialized. In the case of medicinal plants for example many species that are used on household scale are widely available weeds that grow close to the homestead and harvest does not seem to harm these medicinal plant populations (Agelet et al., 2000; Stepp and Moerman, 2001; Stepp, 2004; Towns et al., 2014). Commercial trade, on the contrary, is often more associated with the extraction of primary forest species and is considered to be one of the major threats to wild medicinal plant populations (Marshall, 1998). Rapid urbanization in combination with a growing interest in wild plant resources can stimulate demands and drastically increase harvesting pressure on local plant populations (Cunningham, 1993; Schippmann et al., n.d.). How resilient or susceptible plant species are to harvesting pressure depends on several factors, such as geographic distribution, habitat specificity, local population size, growth rate, plant part used and life form. Another factor not to be overlooked is the extent to which plant populations are pressed by other factors, like habitat loss and changes in land use, since these aggravate the total pressure on plant populations.

Non-timber forest products (NTFPs), to which both medicinal plants and edible orchids belong, can be a valuable source of income for communities, especially in developing countries. Case studies have shown that NTFP harvest can affect ecological processes from an individual level to community scale. The life history of the plants and the plant parts harvested influence the tolerance of species to wild harvesting (Ticktin, 2004). Some wild-harvested NTFPs are commonly available, but there are many wild-harvested plants that are rare or even threatened on local levels or that are becoming exceedingly rare under the harvesting pressure.

In parts of Asia and Africa, the harvest of terrestrial orchids for traditional dishes, has led to rapid declines in local terrestrial orchid populations and has given rise to scarcity-driven cross-border trade (Davenport and Ndangalasi, 2003; Ghorbani et al., 2014). Another example comes from South America, where a survey in the Mexican state of Veracruz has revealed that several national, and even regional, endemic wild epiphytes are harvested for the horticultural (Flores-Palacios and Valencia-Díaz, 2007). These are only a few of the numerous examples of concern regarding the sustainability of commercialized trade in wild-harvested plants (Ticktin, 2004).

Harvesting and trade are, in most countries, regulated on national and international level. Nevertheless, reinforcements of these regulations have proven to be challenging. Despite the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES, 2014) the ivory trade is still booming (Wasser et al., 2007), as is the cross-border trade of protected orchids, and medicinal plants and animals (e.g.(Davenport and Ndangalasi, 2003; Coghlan et al., 2012; Ghorbani et al., 2014; Veldman et al., 2014). An important aspect of monitoring international trade in endangered species is fast, cost-effective and unambiguous identification of the species in trade, independent of their traded state. Establishing which species are traded and in what quantities, is a prerequisite to decide, in combination with population data, which species to prioritize for conservation and how to regulate trade. Regulation of trade should also take into account sustainability of the species and the people whose livelihoods depend on their trade (Schippmann et al., n.d.; Hamilton, 2004). Identifying which plants are traded can sometimes be done based on morphology, but in some cases, plants or plant parts are traded in morphologically undistinguishable manners, such as sterile leaves, barks, roots, tubers and powders. To identify morphologically challenging substrates people have been employing molecular methods for the identification of species which were previously impossible to identify (e.g., Baker et al., 2010; Kool et al., 2012; Coghlan et al., 2012; Galimberti et al., 2013).

1.3 Medicinal plants

To date a large part of the world population uses medicinal plants for their primary healthcare (World Health Organization, 2013), and it was reported that medicinal plant use is rising in developed countries (Schippmann et al., n.d.). Certain common medicinal plant species are cultivated, but the majority of species and volume is harvested from the wild (Schippmann et al., n.d.; Hamilton, 2004).

In Tanzania medicinal plants are an important part of the local healthcare in both urban and rural areas (Marshall, 1998; Otieno et al., 2001; de Boer et al., 2005; Otieno et al., 2006). It is estimated that approximately 25% of the ca. 11.000 Tanzanian vascular plants are used medicinally, although it is not clear how this percentage is calculated (ITM, 2016). More conservatively, we could rely on the global estimated percentage of medicinal flora, which is 10-18% of the total flora (Schippmann et al., n.d.). Several studies have been done on medicinal plants used in Tanzania. An extensive survey performed in northeastern Tanzania in the Handeni, Lushoto, Korogwe, Same and Tanga districts, listed a total of 153 medicinal plant species used by traditional healers (Hedberg et al., 1982, 1983a, 1983b). In 1998 a review on traditional medicine trade in East and Southern Africa was published, identifying 98 medicinal plant species traded on markets in the Dar-es-Salaam and Tanga region (Marshall, 1998). More recently a study of species traded on the medicinal plant markets in Dar-es-Salaam has been done, listing ca. 200 ethnospecies – vernacular names given to local species entities (Nahashon, 2013).

However, what has been lacking so far is a quantitative market survey, giving an overview of both species diversity and the volumes traded commercially per species at the Tanzanian medicinal plant markets, as well as accurate and reproducible identifications of marketed species based on reference vouchers. The papers cited above all provide only qualitative insights into medicinal plants, and the recent market survey performed by Nahashon (2013) made identifications based on literature only, without verifying these identifications with voucher material. A major issue with matching ethnospecies to scientific species based on literature is that local species concepts do not necessarily correspond to our scientific species concept. One ethnospecies might for example refer to all available species within a certain genus (The Plant List, 2018). This makes it hard to determine exactly which species are available on the market and which subsequent species could be at risk of overharvesting.

1.4 Edible orchids

Orchidaceae is one of the most diverse flowering plant families, comprising an estimated 27,801 species in 899 genera (The Plant List, 2018). Economically they are best known for their horticultural use and for vanilla. Less known is that in several countries in Asia and Africa terrestrial orchid tubers are consumed in large quantities as well. An example is *salep* from present-day Turkey (Kasparek and Grimm, 1999; Sezik, 2002) and Greece (Kreziou et al., 2015), a use that spread to Europe in the 17th and 18th century. Records of the Swedish Academy show for example that *salep* was already mentioned as early as 1764 in Swedish literature (Svenska Akademien, SOAB., 2018).

In Zambia terrestrial orchids are used as an ingredient in a traditional relish called *chikanda*. *Chikanda* was originally a poor man's food eaten by tribes in Northern Zambia in times of food scarcity (Richards, 1939). It consists of ground orchid tubers, peanuts, ashes or baking powder and some chili pepper (Bingham, 2007). Nowadays *chikanda* is a popular snack throughout the country, and trade in orchid tubers used for *chikanda* has commercialized. In the first papers mentioning the thriving *chikanda* trade/and exponential growth in *chikanda* consumption, the observation was already made that this could pose serious threats to local orchid populations. Several species have been recorded to be used for *chikanda*. Initially only the use of *Disa robusta* Rendle. was recorded (Cribb and Leedal, 1982), but later observations showed that more and more species within the genera *Disa*, *Satyrium* and *Habenaria* were used (Bingham and Smith, 2002; Davenport and Ndangalasi, 2003; Bingham, 2004; Nyomora, 2005; Bingham, 2007; Challe and Price, 2009; Hamisy, 2010). It is now thought that the tubers of species in these genera are harvested indiscriminately (Bingham, 2007). Moreover, although tubers were originally harvested locally in Zambia, they are now said to be imported from several surrounding countries, because of local depletion (Davenport and Ndangalasi, 2003; Veldman et al., 2014). One of the countries from which the orchids are currently imported is Tanzania, and it is home to a vast number of terrestrial orchid species belonging to the favored *chikanda* genera. The Southern Highlands in southwestern Tanzania are especially rich in orchid diversity and populations. In a survey done approximately fifteen years ago, it was shown that an alarming amount of 2.4 – 4.1 million orchids tubers is harvested from the wild on a yearly basis (Davenport and Ndangalasi, 2003), and a recent survey confirms these numbers are still similar to-date (Veldman et al., 2014). Harvesting areas contain many orchid species and harvesters are known to target species indiscriminately, and it is estimated that as many as 85 Tanzanian species are at risk of overharvesting (Davenport and Ndangalasi, 2003). This number, however, seems to include only the species for the preferred *chikanda* genera: *Disa*, *Satyrium* and *Habenaria*. If harvesters are now also targeting species from *Brachycorythis*, *Roeperocharis* and *Eulophia*, the number of species possibly at risk of overharvesting is even higher (Challe and Price, 2009; Hamisy, 2010). Studies done on *chikanda* so far have made identifications based on morphology, by either joining the collectors in the field or by growing the entire plant from the tubers. However, no analyses have been done on *chikanda* to see which species are actually present, and no quantitative surveys have been done do determine which species are sold at the market. The processed state of *chikanda*, a baked mixture of ground orchid tubers and other plant ingredients, has made it impossible to determine which species have been used in it production. Innovations in high-throughput sequencing and amplicon metabarcoding have made it possible to study species diversity in *chikanda* using DNA sequence data (Veldman et al., 2017).

2 Methods

2.1 Research areas

The United Republic of Tanzania is located in Eastern Africa, and borders the Indian Ocean in the East. With a population of over 51 million and an annual population growth rate of 2.6%, Tanzania is one of Africa's fastest growing countries (CIA, 2018a). Tanzania is a country of low development on the Human Development Index, but regional assessments show that the Dar-es-Salaam region is of medium HDI development (UNDP, 2016). The Republic of Zambia is a landlocked country in south-eastern Africa, bordering Tanzania in the north-east, Angola and the Democratic Republic of Congo in the east, Zimbabwe and Mozambique in the south and Malawi in the east. The country has a population of 16 million and the highest urbanization levels in entire Africa, with the cities of Lusaka, Ndola, Kitwe and Mufulira showing exceptionally high densities. Zambia is considered a lower-middle income country, but unemployment and rural poverty are still considered significant problems (CIA, 2018b).

2.1.1 Medicinal plants – Dar-es-Salaam and Tanga

The medicinal plant research in this thesis is focused on two coastal urban areas in Tanzania, Dar-es-Salaam and Tanga. Dar-es-Salaam is the country's main urban area and commercial center, with approximately 30% of the total population living there. Marshall (1998) indicates that there are two main medicinal plants markets in Dar-es-Salaam: Kariakoo and Kinondoni. However a more recent survey of commercial trade in medicinal plants has identified some additional locations in the city where medicinal plants are traded (Nahashon, 2013). Supplies are said to come from different regions in Tanzania, such as Handeni, Korogwe, Pangani, Shinyanga and Sumbawanga districts, as well as the Uluguru and Usambara mountain regions (Marshall, 1998). Several of these harvesting regions are located in or close to two East African biodiversity hotspots: the Eastern Arc and Coastal Forests of Tanzania and Kenya, which are reported to be home to about 1500 endemic plant species (Myers et al., 2000). Another urban area located closely to these two biodiversity hotspots is Tanga. Like Dar-es-Salaam, Tanga has a flourishing medicinal plant trade, with approximately 50 medicinal plant traders (Marshall, 1998). Medicinal plant traders from Tanga indicated Muheza, Amani, Korogwe and Handeni districts as collection sites for their medicinal plants (Marshall, 1998; McMillen, 2008).

2.1.2 Edible orchids – Southern Highlands and Zambia

Chikanda consumption originated from the Bemba people in northern Zambia and in this region the *chikanda* trade is flourishing. Additionally due to the urbanization which is taking place, the capital city Lusaka has become the center of Zambian *chikanda* trade, with most end consumers. Yet additional trade centers are Kitwe, Ndola and several cities along the TAZARA highway. In a survey done on the Soweto market in Lusaka, informants mentioned Tanzania's Southern Highlands as one of the main sources for *chikanda* tubers. Orchid harvesters are forced to obtain an increasing amount of their supplies from abroad, since the Zambian orchid resources have become rather depleted (Davenport and Ndangalasi, 2003; Bingham, 2007). The Kitulo Plateau is part of the Southern Highlands and is recognized as an area of large biological diversity. Characteristic for the area are the high-altitude grasslands with unique flora and fauna (Cribb and Leedal, 1982). They house approximately 85 terrestrial orchid species within the *Disa*, *Satyrium* and *Habenaria* genera of which 31 species are considered to be endemic to Tanzania (Davenport and Ndangalasi, 2003). In 2004 Kitulo National Park was established with the specific purpose of protecting terrestrial orchids against the intense harvesting pressure from *chikanda* trade (Davenport and Bytebier, 2004), it has been reported however, that terrestrial orchids are still poached from the area (Veldman et al., 2014).

The Tanzanian border town Tunduma has been identified as trade hub for the cross-border *chikanda* trade between Tanzania and Zambia. Middleman and vendors gather *chikanda* tubers from different parts of Tanzania and even Northern Zambia and sell them to wholesalers and retailers that will transport them to markets in Zambia (Davenport and Ndangalasi, 2003; Bingham, 2007).

2.2 Quantitative market research and interview methods

Quantitative market surveys are a successful method of assessing a countries medicinal flora as well as local health concerns (Cunningham, 2001; Williams, 2003; Bussmann et al., 2007; van Andel et al., 2008; Quiroz et al., 2014; Towns et al., 2014). Quantitative market research starts with exploring the local markets and familiarizing with the market vendors and traded goods. After this initial reconnaissance, one can proceed with interviewing market vendors about which plants are traded, harvesting sources, available stocks and perceived availability of the traded plants. In addition, full stall inventories can be made for several market stalls, in which reference vouchers are collected for each species traded and the available stocks per stall is recorded in number of units, average weight per unit and unit price. After several market stalls inventories are made, ideally a representative number for the rest of the market, an extrapolation can be made to the entire market, allowing for total estimates on diversity and volumes traded (Martin, 2004; van Andel et al., 2012; Quiroz et al., 2014; Towns et al., 2014).

Such interviews and inventories with vendors and collectors give an indication of the availability of the traded species, and make it possible to identify species which might be at risk of overharvesting. Identifying species in need of conservation efforts is a first step in ensuring a sustainable supply of herbal medicine in the future.

2.3 DNA barcoding

A little over a decade ago Hebert et al. (2003) coined a new term in the field of species identification: DNA barcoding. The idea of DNA barcoding would be that each species is identifiable using one relatively short DNA fragment, which should be variable between species and conserved within species. This variable region should be flanked by conserved sites for primer binding, allowing the use of a single primer pair to amplify the targeted region for all species. By amplifying this region and matching it to a reference database, it should be possible to identify vouchers or samples up to species level and to even place new samples within the right taxon.

For animals, cytochrome c oxidase I (COI) was proposed as a barcoding region (Hebert et al., 2003). In subsequent years, this marker was used extensively for animal species, and it is now more or less universally accepted as the first marker of choice (Hanner, 2009). In plants, determination of a DNA barcode was less straightforward. Several regions in the plastid genome (e.g., *rbc*L, *mat*K, *psb*A-*trn*H) and one in the nuclear genome have been proposed (nrITS), but no single region seems to have sufficient discriminatory power to serve as a DNA barcode by itself (Hollingsworth et al., 2009). Although the debate has not been concluded, the use of combined loci as a plant barcode has been proposed. The most suitable core plant barcodes were deemed *rbcL* and *mat*K, which can be complemented by other markers, such as ITS and *psb*A-*trn*H (Hollingsworth et al., 2009). A recent survey has shown that ITS has the highest discriminatory power of these four barcoding regions, with a success rate of 62.7% (Li et al., 2011). This finding is amongst others supported by field studies on molecular identification of cycads (Sass et al., 2007), Amazonian trees (Gonzalez et al., 2009) and medicinal plants traded on Moroccan markets (Kool et al., 2012). Research done by Chen et al. (2010) even reported a success rate of 92.7% for species level identification with ITS2, based on identifications for 6.600 samples from 4.800 species belonging to 753 distinct genera.

Besides the availability of a discriminatory marker set, another essential component for successful and reliable species-level identification through DNA barcoding is a reference database with sequences for all species of interest. However, although a huge amount of sequences is available online today, we are still far from having sequences for all existing species (Benson et al., 2012). This makes it often necessary for specific projects, to compile their own reference database with sequences of expected species and their close relatives (e.g. Sass et al., 2007; Gonzalez et al., 2009; Kool et al., 2012; Yan et al., 2015).

DNA barcoding generally makes use of Sanger sequencing to obtain the required barcoding regions. When analyzing vouchers or samples with only one species, this approach functions well. However, when it comes to mixtures of multiple species Sanger sequencing alone does not suffice. Recently more advanced sequencing methods such as high-throughput sequencing have been employed to analyze samples containing a DNA of multiple organisms for their species composition. Examples are studies on ancient lake sediments (Boessenkool et al., 2014), mammoth diet during the Middle Pleniglacial (van Geel et al., 2008) and historical vegetation reconstruction (Willerslev et al., 2014). This metabarcoding approach has also been used in the authentication of herbal medicine and food products. Studies on traditional Chinese medicine have for example been able to detect the presence of dozens of species from different plant families in medicinal mixtures, as well as certain endangered animal species (Coghlan et al., 2012, 2015), DNA metabarcoding using nrITS has been able to detect substitution and adulteration of species in various European herbal products (Raclariu et al., 2017a, 2017b, 2017c) and the provenance of honey could be authenticated using metabarcoding of pollen (Richardson et al., 2015).

2.4 Species identification

To this day one of biology's most disputed concepts is a species. The traditional biological species concept as described by Mayr (1942) is as followed: "Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups", but this has in practice led to several conundrums. Besides the biological species concept many alternative species concepts have been proposed, all describing the subject from a different angle (De Queiroz, 2007). It seems that the common denominator in all these species concepts is the evolutionary perspective that species are segments of metapopulational lineages, whereas each concept seems to propose additional requirements that should be met by individuals in order for them to be considered the same species. Examples are: reproductive isolation (isolation species concept), occupation of a distinct niche (ecological species concept) and sharing derived character states (monophyletic species concept). Identification of species using DNA barcoding makes use of the genetic or phylogenetic species concepts, where molecular operational taxonomic units are defined based on sequence heterogeneity. Each species concept has its own advantages and pitfalls and it is essential to keep these in mind when applying species concepts.

2.5 Research ethics

The research was conducted in accordance with the guidelines in the Code of Ethic of the International Society of Ethnobiology (International Society of Ethnobiology, 2006). Collaboration was initiated with the Tanzanian Institute for Tradition Medicine (ITM), part of the Muhimbili University of Health and Allied Sciences (MUHAS), with whom a Material Transfer Agreement was drawn up. The research project was registered at the Tanzania Wildlife Research Institute (TAWIRI) and research permits were obtained from the Tanzania Commission for Science and Technology (COSTECH). Residence permits were attained through the Tanzanian Department of Immigration Services. In case of collection in a national park we were granted permission from the Tanzania National Park Authorities (TANAPA).

Before conducting interviews, the nature of our research and our intended outcomes were explained to participants and written prior informed consent (PIC) was obtained. Duplicates of all samples were deposited at the herbarium of the Institute of Traditional Medicine (ITMH) and Naturalis Biodiversity Center (L) in the Netherlands. Export permits were obtained through the Phytosanitary Section of the Ministry of Agriculture and Food Security and from TAWIRI in case of CITES-listed material.

3. Aims

The aims of my doctorate are **I)** to investigate whether species that are traded on local markets can be identified using molecular methods such as DNA barcoding and high-throughput sequencing, **II)** to map harvest and trade of Tanzanian medicinal plants and Tanzanian and Zambian edible orchids and **III)** to identify conservation issues arising from wild-harvesting of medicinal plants and edible orchids.

Paper I describes how amplicon metabarcoding can be used to determine species composition of a highly processed mixture containing multiple terrestrial orchid species, and sheds light on which species are used for the traditional Zambian dish *chikanda*. **Paper II** identifies the non-powdered leaves, fruits and seeds that are traded on the Kariakoo market in Dar-es-Salaam, quantifies trade and evaluates conservation issues arising from the trade in these wild-harvested medicinals. **Paper III** identifies powdered as well as non-powdered medicinal plants traded on Dar-es-Salaam and Tanga markets using a tied approach combining literature, morphology and DNA barcoding with three different markers. **Paper IV** builds upon the identifications made in paper III and quantifies the trade in medicinal plants and identifies possible conservation issues connected to this trade. **Paper V** provides an overview of quantitative markets surveys done in three Zambian provinces with a flourishing *chikanda* trade. Tubers bought on the local markets were identified using DNA barcoding in a phylogenetic context and species most susceptible to overharvesting were identified.

Results and discussion

Paper I: Metabarcoding of *chikanda* cake

Veldman, S., Gravendeel, B., Otieno, J.N., Lammers, Y., Duijm, E., Nieman, A., Bytebier, B., Ngugi, G., Marcos, F., van Andel, T.R., de Boer, H.J. (2017). High-throughput sequencing of African chikanda cake highlights conservation challenges in orchids. *Biodiversity and Conservation*, 1-18.

Chikanda is a traditional Zambian dish consisting of ground orchid tubers, peanuts, chili powder, baking soda and salt, which are mixed, boiled and subsequently baked into a cake on a stove (Bingham, 2004; Veldman et al., 2014). Originally terrestrial orchids were only used in Zambia and surrounding countries in times of famine and only two orchid species were reported to be used: *Disa robusta* and *Satyrium buchananii* (Cribb and Leedal, 1982; Davenport and Ndangalasi, 2003). In recent years the dish has become more popular and species are harvested indiscriminately. To meet the Zambian demand for *chikanda*, terrestrial orchids are imported from surrounding countries, such as Tanzania. Although as many as 85 orchid species could be at risk of extinction in Tanzania because of *chikanda* trade, it is unclear what species are used specifically (Davenport and Ndangalasi, 2003; Bingham, 2007). In this paper the species composition of six ready-made *chikanda* samples is analyzed using amplicon metabacoding. Analysis of ITS1 and ITS2 sequences showed the presence of 36 plant species, including 17 different orchid species. *Disa* species were present in all of the samples, *Satyrium* in five out of six and *Habenaria* was only present in one of six *chikanda* samples. Besides these ingredients, the expected peanut and chili were detected, as well as several adulterants or processing contaminants such as mango, sweet potato and okra. This study of a limited number of *chikanda* samples shows that amplicon metabarcoding using ITS2 is a powerful method to identify orchid species in a highly processed product like *chikanda* and revealed that a substantial amount of orchid species from three different genera is being harvested in Tanzania for *chikanda*. Even if none of the detected species are on the IUCN Red List (IUCN, 2018), harvesters report that *chikanda* orchids becoming more difficult to obtain, indicating that commercial harvest puts a serious threat on local orchid populations (Veldman et al., 2014). Identifying the diversity of orchid species used for *chikanda* allows for targeted conservation measures for those orchids that are most at risk of overharvesting.

Paper II: Medicinal leaves, seeds and fruits at Kariakoo

Posthouwer, C., **Veldman, S.**, Abihudi, S., Otieno, J.N., van Andel, T.R., de Boer, H.J. (2018). Quantitative market survey of non-woody plants sold at Kariakoo Market in Dar-es-Salaam, Tanzania. *Journal of ethnopharmacology,* 222, 280-287.

In Tanzania a large part of the population relies on traditional medicine for their primary healthcare. Kariakoo ward in Tanzania's main economic center Dar-es-Salaam harbors a flourishing medicinal plant market (Marshall, 1998). Besides many powders, barks and roots sold at the medicine market a substantial amount of dried herbs, leaves, fruits and seeds is traded (Nahashon, 2013). Markets surveys so far have not vouchered the medicinal plants sold at the markets and have instead identified species traded mainly based on the vernacular names of the plant products. One vernacular name can, however, refer to multiple scientific species and multiple species can share the same vernacular name (Berlin, 1973; Martin, 2004; Kokwaro, 2009), which makes it challenging to determine what species are traded and in what quantities. This study maps the diversity of species traded, quantifies the annual amount in which they are sold and looks at the associated conservation issues arising from the wild-harvest of these plants. Additionally it documents the major use categories and the salient health issues associated with the plants. The 71 encountered medicinal plant products were identified as 62 to 67 species belonging to four different plant families. Annual trade volumes are at least 30 tons, worth 200,000 USD and the most frequently encountered plants are *Sclerocarya birrea* (A.Rich.) Hochst. (Anacardiaceae), *Myrothamnus flabellifolia* Welw. (Myrothamnaceae) and *Suregada zanzibarensis* Baill. (Euphorbiaceae). The plants sold at the market were mainly used to treat women's health issues, digestive disorders and ritual purposes. None of the species encountered were on the IUCN Red List, but *Aloe* sp. and *Vanilla* sp. are both CITES-listed. Vendors indicated that some plant products were increasingly difficult to obtain, but overall no large conservation issues seem to arrive from the trade in dried leaves, herbs, fruits and seeds.

Paper III: DNA barcoding of Tanzanian medicinal plants

Veldman, S., Ju, Y., Abihudi, S., van Andel, T., Otieno, JN., de Boer, HJ. Identifying the unidentifiable – DNA barcoding of medicinal plants traded on Tanzanian markets. (Manuscript).

Applied ethnobotanical studies such as quantitative market surveys need reliable methods to identify the traded taxa up to species level in order to quantify trade and determine if there are associated conservation issues. When the marketed products are fresh fruits, seeds or leaf bundles it is often possible to morphologically identify voucher specimens bought on the markets (van Andel et al., 2012; Towns et al., 2014; Quiroz et al., 2014). In case of medicinal plants traded in Tanzanian cities, however, many traded products lack morphological characters needed for identification, since they are traded as powders, shredded material, roots or barks (Nahashon, 2013). Moreover, local ethnospecies often do not correspond one-to-one with scientific species (Otieno et al., 2015). This study used a combined approach of morphology, literature and DNA barcoding to identify species traded on the local medicinal plant markets. A total of 873 medicinal plant samples corresponding to 452 ethnospecies were purchased on the Dar-es-Salaam and Tanga markets. DNA barcoding managed to identify 112 plant products that could not be determined by literature and morphology up to species- to family-level. Of the barcoding markers used *rbcL* proved to have the highest amplification success, whereas nrITS showed the most successful species-level identification success. In total 240 plant products could be identified, showing 32 cases of overdifferentiation and 48 of underdifferentiation. Although not all specimens could be identified using DNA barcoding, it has proved a useful method to identify specimens that are not possible to identify using morphology or literature. Additionally it can be used to verify or fortify these identifications, especially in the case of morphologically unidentifiable specimens and specimens which have over- or underdifferentiated ethnospecies they correspond to. Moreover, DNA barcoding can be used to check for adulteration and substitution and thus for authentication of traditional medicine sold on local markets. Using a tied approach of identification methods can shed light on the diversity of species traded on local markets, which can subsequently be used as input for quantitative market research and the evaluation of possible sustainability issues associated with commercialized trade in wild-harvested plants.

Paper IV: Quantification of medicinal plants sold on Tanzanian markets.

Veldman, S., Abihudi, S., Posthouwer, C., van Andel, T., Otieno, JN., de Boer, HJ. Trade in Tanzania's threatened trees – a quantitative market survey of medicinal plants used in Dar-es-Salaam and Tanga. (Manuscript).

Tanzania has a thriving medicinal plant trade, which is solely based on the harvest of wild plant resources (Marshall, 1998; McMillen, 2008). Many of the plants are dried or powdered and often lack sufficient morphological characters for species-level identification (Nahashon, 2013). It is thus unclear which exact species are traded and in what amounts. This study provides an overview of species traded in the main Tanzanian coastal trade hubs Dar-es-Salaam and Tanga, quantifies the annual sales volumes of these plants and gives an overview of the possible conservation issues arising from the wild-harvest of Tanzanian medicinal plants. Based on the interview data from 46 questionnaires an overview was compiled of the plants most frequently mentioned by the market vendors as well as a list with plants that were considered to become more difficult to obtain. A total of >850 single ingredient samples was collected. The main health categories for which these plants are reported to be used are women's health, ritual purposes and respiratory problems. In addition to vouchers from the plants sold on the market, reference herbarium vouchers were made in the field together with a medicinal plant collector for the twenty most frequently mentioned plants. Results from the field indicate that there are high harvesting pressures on some of the most commonly traded medicinal plant species. The top five plants that are considered to be becoming scarcer are *Zanha africana* (Radlk.) Exell. (Sapindaceae), *Zanthoxylum* sp. (Rutaceae), *Warburgia* sp.. (Canellaceae), *Allophylus* sp. (Sapindaceae) and *Cassia abbreviata* Oliv. (Leguminosae). Alarmingly, four out of these five plants also figure in the list of most frequently mentioned plants and several vendors indicate they each sell up to 200kg of these species per month. *Warburgia elongata* is included on the global Red List as endangered, as well as several *Zanthoxylum* species. From these plants the bark, roots and branches are used for medicine, parts which are often harvested unsustainably. In order to conserve local medicinal plant populations and ensure a sustainable herbal medicine supply, it is essential to look at sustainable harvesting strategies as well as cultivation possibilities in collaboration with the harvesters, middlemen and vendors.

Paper V: Identification and conservation of orchids used for *chikanda*.

Veldman, S., Kim, S-J., Gravendeel, B., Wightman, N., Vinya, R., Mpatwa, G., Chuba, D., Bone, R.E., Bytebier, B., Ngugi, G., Marcos, F., van Andel, T.R., de Boer, H.J. Trade in Zambian edible terrestrial orchids - molecular identification reveals the use of previously undocumented orchid taxa for chikanda. (Manuscript).

In Zambia the consumption of the national delicacy *chikanda* has led to the depletion of local orchid populations (Bingham, 2007). Where *chikanda* originally was only consumed locally and in times of famine, it has now become popular throughout the country, which has spurred a flourishing international trade in orchid tubers. Terrestrial orchids from six different genera are thought to be used for *chikanda* and are harvested from Zambia as well as neighbouring countries (Davenport and Ndangalasi, 2003; Veldman et al., 2014). Currently it is impossible to determine what species are sold on local markets, since the marketed tubers have comparable morphology. This study applies DNA barcoding to identify tubers traded on Zambian markets to determine the diversity of orchid species traded and to provide insight in *chikanda* trade in the areas where most of the chikanda trade is taking place. Tubers collected on 25 different markets in three Zambian provinces were analysed using DNA barcoding with the standard markers *rbcL* and *matK* as well as nrITS. The core landplant barcoding markers *rbcL* and *matK* yielded family and genus level identifications, whereas nrITS often showed a higher variability between species that allowed for species level identifications. In total 16 orchid species belonging to six different genera were identified from the traded tubers. The most frequently identified species were *Disa robusta, Satyrium buchananii,* and *Platycoryne crocea*. Taking this study and the previous literature into account this suggests that 49 orchid species are used for *chikanda*, of which hardly any are mentioned on local or global Red Lists. Moreover, orchids continue to be traded internationally, which should be strictly regulated under CITES-legislation. Participants in our study confirmed that *chikanda* orchids become increasingly difficult to obtain, indicating unsustainable harvest. Knowing what orchid species are used for *chikanda* allows us to take targeted conservation measures in collaboration with local authorities and the people involved in *chikanda* trade, attempting to both conserve the wild orchid populations as well as the Zambian tradition of *chikanda* consumption.

Concluding remarks

This thesis consists of five papers, which aim to map harvest and trade of Tanzanian wild-harvested medicinal plants (**paper II**, **III** and **IV**) and edible orchids (**paper I** and **V**) and to look at possible conservation issues arising from commercialized trade in these species. Since the studied medicinal plants and edible orchids have in common that they are often impossible to identify based on morphology alone, a DNA barcoding and metabarcoding approach has been employed to identify what species are traded at local markets and to investigate to what extend local species concepts are in congruence with scientific species concepts.

In **paper I** metabarcoding of Tanzanian *chikanda* cake shows the presence of 17 different orchids species belonging to the genera *Disa*, *Satyrium* and *Habenaria*, as well as 19 other plant species. Although barcoding with the nuclear ribosomal marker ITS performs fairly well, an expansion of the Tanzanian and Zambian orchid reference sequence database is highly recommended. Additionally, **paper I** shows that people involved in *chikanda* trade report that it is more and more difficult to obtain supplies and although it seems that not all Tanzanian orchid species belonging to these genera are at risk of overharvesting, measures should be taken to ensure a more sustainable use of the local *chikanda* orchid resources. These findings are further supported by the results from **paper V**, which shows that at least 16 different orchid species, belonging to six genera are traded as *chikanda* on Zambian markets. This brings the total amount of orchid species reported to be used for *chikanda* to 49 in eight different genera, which confirms the notion that harvesters do not limit themselves to the originally preferred orchid species and are now targeting orchids indiscriminately and conservation measures should be implemented. In **paper V** we also show that the two chloroplast markers *rbcL* and *matK* can be used to identify *chikanda* vouchers up to family or genus level, and that nrITS can be used in many cases for species level identification. Reliable species level identifications would, however, be aided by an expansion of the reference sequence database and more specifically by the inclusion of multiple representatives of all species occurring in southern Africa.

Paper II shows that annually nearly 31 tonnes of fresh and dried medicinal leaves, seeds and fruits are sold at Kariakoo market in Dar-es-Salaam, with an estimated value of 200,000 USD. The most frequently sold species are

Suregada zanzibariensis, *Cassytha filiformis, Pupalia lappacea, Ehretia amoena* and *Solanum* sp. and in total 62 to 67 different species belonging to 41 plant families are traded. The main health concerns for which the plants are used are digestive disorders, women's health and ritual purposes. It seems that the majority of these medicinal plants do not have associated sustainability issues since most are harvested from disturbance areas and non-detrimental harvesting practices are applied. The bulk of medicine sold at Kariakoo market consists of barks, roots, branches and powders, which account for an estimated 180 tonnes of annually traded material. In **paper III** it was determined what species are sold at medicinal plant markets in Dar-es-Salaam and Tanga, and to what extent local species concepts corresponded with scientific species concepts. We attempted to identify more than 850 market samples using a tied approach of DNA barcoding with *rbcL*, *matK* and nrITS, morphology and literature. In total, 509 identifications could be made corresponding to at least 175 species, 124 genera and 65 plant families, and several cases of over- and underdifferentiation were detected. Similar to **paper V** nrITS proved to be the most discriminative marker rendering the majority of species level identifications. Moreover, also in case of Tanzanian medicinal plants it is essential to expand the reference sequence library with multiple individuals per species and as many species per genus of the plants occurring in Tanzania. **Paper IV** uses the identifications made in **paper III** to quantify trade in the morphologically unidentifiable plants sold on Tanzanian markets, such as barks, roots and powders. Four of the most frequently mentioned plants are also mentioned by vendors as medicinal plants that are becoming increasingly difficult to obtain.

Overall this thesis shows that DNA barcoding has significant potential as a rapid identification method for morphologically unidentifiable specimens, with nrITS showing the highest discriminatory power of the three markers that were used. In order to improve identification success using DNA barcoding it is essential to expand sequence reference databases, since African species seem to be severely underrepresented. Inclusion of multiple individuals per species would allow for accurate estimates of inter- and intraspecific variation and thus a more accurate species-level identification threshold. Last but not least, this thesis shows that wild-harvested medicinal plants and edible orchids are traded in large quantities in Tanzania and Zambia and that commercialization puts local useful plant populations under serious harvesting pressure.

Svensk sammanfattning

Den här avhandlingen kartlägger skörd och handel av medicinalväxter och ätbara orkidéer i Tanzania och Zambia, samt undersöker möjliga bevarandeproblem kopplade till detta. Genom hela den mänskliga historien har växter använts för en rad olika ändamål. Växter har försett människor med mat, medicin, timmer, tyg och bidragit till olika typer av ekosystemtjänster. När de vilda växterna användes i mindre skala var det inga problem, men nu när användningen av vildskördade nyttoväxter har kommersialiserats, kan det uppstå bevarandeproblem för lokala växtpopulationer. De nyttoväxter som jag har undersökt säljs på lokala marknader i former som gör dem morfologiskt oidentifierbara. Därför använder jag en molekylär metod som kallas DNA-streckkodning för att komma fram till vilka växtarter det är som faktiskt säljs. Om vi vet vilka växter som säljs, och i vilka mängder, kan vi undersöka om det finns bevarandeproblem på grund av handeln och, om nödvändigt, vidta åtgärder.

I **artikel I** använde vi DNA-metastreckkodning för att identifiera orkidéarter som används till den traditionella zambiska delikatessen *chikanda* – en orkidékaka gjord av malda jordnötter, orkidéknölar och chilipulver. Ursprungligen användes bara en eller två orkidéarter till chikanda: *Disa robusta* och ibland *Satyrium buchananii*, men i takt med att chikanda har blivit populär i hela Zambia används fler och fler arter av orkidéer. I kakorna som jag har analyserat fanns det åtminstone 17 olika orkidéarter från släktena *Disa*, *Habenaria* och *Satyrium*, samt 19 andra växtarter, bl.a. jordnöt, sötpotatis, mango, kassava och okra. Människor som är inblandade i orkidéhandeln berättar att det har blivit svårare att hitta orkidéerna i det vilda och det rapporteras att orkidéer i allt större utsträckning importeras från olika grannländer för att möta den ökade efterfrågan. Det här bekräftas i **artikel V** där orkidéknölar på zambiska marknader undersöktes. Målet med denna studie var att undersöka vilka orkidéarter som säljs som chikanda i Zambia, om de kan identifieras med hjälp av DNA-streckkodning, var orkidéerna skördas och om det har uppstått bevarandeproblem på grund av handeln. Undersökningar och intervjuer genomfördes på 25 olika marknader i tre zambiska provinser och 48 chikandaprover köptes. Med hjälp av DNA-streckkodning var det möjligt att identifiera 16 orkidéarter från sex olika släkten. Det visade sig att de två kärnstreckkodsmarkörerna som vanligen används för landväxter kunde användas för att identifiera växter på familj- och släktesnivå, medan det var möjligt att skilja orkidéer åt på artnivå med streckkodsmarkören nrITS. För att få mer pålitliga artidentifieringar är det viktigt att utvidga referensdatabasen med sekvenser, eftersom inte alla markväxande orkidéer finns med i databasen och många av de arter som är med bara har en enda representant. Detta gör det svårt att bestämma ett gränsvärde för artbestämning med DNA-streckkodning. Det uppskattas att totalt 49 olika orkidéarter används till chikanda och bara ett fåtal av dessa finns med på nationella och internationella listor över fridlysta arter. I Zambia berättar människor inblandade i chikanda-handel att det blir allt svårare med orkidéleveranserna, vilket tyder på att ohållbar skörd äger rum och att bevarandeåtgärder bör vidtas.

De övriga artiklarna i avhandlingen behandlar handel med tanzaniska medicinalväxter. I Tanzania, som i flera afrikanska länder, använder en stor del av befolkningen medicinalväxter för egenvård. Människor samlar dessa medicinalväxter i sitt närområde, får dem från traditionella läkare eller köper dem på en medicinalväxtmarknad. Nästan alla medicinalväxter är vildskördade och kommersiell handel är ett av de största hoten mot de vilda populationerna. För att veta vilka växter som hotas på grund av handeln är det essentiellt att veta vilka växter som säljs på marknader, i vilka mängder och var de skördas. **Artikel II** beskriver en undersökning av alla icke-pulveriserade och ej vedartade medicinalväxter som säljs på Dar-es-Salaams största medicinmarknad Kariakoo. Totalt hittade vi mellan 62 och 67 arter från 41 olika växtfamiljer av vilka blad, frukter eller frön såldes. Handeln med dessa växter uppgår till 31 ton per år vilket motsvarar 200 000 USD. *Suregada zanzibariensis*, *Cassytha filiformis, Pupalia lappacea, Ehretia amoena* och *Solanum* sp. är de mest sålda växtarterna, men inget tyder på att dessa växter är hotade på grund av handeln, eftersom skörden sker på ett hållbart sätt. I **artikel III** använde vi DNA-streckkodning i kombination med litteratur och morfologi för att identifiera medicinala rötter, pulver och bark som säljs på marknader i Dar-es-Salaam och Tanga. En stor del av handeln med medicinalväxter äger rum i dessa två städer. Tidigare studier har visat att många av de växter som säljs här skördas i de tanzaniska Eastern Arc Mountains och Coastal Forests som är viktiga ”hotspots” för biologisk mångfald med många endemiska arter. Vi samlade över 850 medicinalväxtprover från olika marknader och försäljare och intervjuade 46 växthandlare. **Artikel III** visar att det fanns åtminstone 175 växtarter från 65 olika växtfamiljer på marknaden och att nrITS fungerade bäst för att identifiera proverna till artnivå. Vi fann även att lokalt använda artavgränsningar inte nödvändigtvis motsvarar våra vetenskapliga. Bland de växter som såldes fanns 32 fall av överdifferentiering där man har flera lokala namn för en vetenskaplig art och 48 fall av underdifferentiering där man har ett lokalt namn som motsvarar flera vetenskapliga arter. Även i det här fallet är det nödvändigt att utvidga referensdatabasen för mer pålitliga artidentifieringar, eftersom inte alla tanzaniska medicinalväxter är med i databasen. **Artikel IV** bygger vidare på identifikationerna och kvantifierar handeln med medicinalväxterna, samt undersöker vilka växter som möjligtvis är eller kan bli hotade på grund av handeln. De fem växter som har blivit svårare att hitta i det vilda är: *Zanha africana* (Radlk.) Exell. (Sapindaceae), *Zanthoxylum* sp. (Rutaceae), *Warburgia* sp.. (Canellaceae), *Allophylus* sp. (Sapindaceae) och *Cassia abbreviata* Oliv. (Leguminosae). De flesta av dessa tillhör också de växter som det handlas mest med. Eftersom skörden av bark, rötter och grenar av dessa medicinalväxter ofta sker på ett ohållbart sätt är det viktigt att försöka hitta mer hållbara lösningar tillsammans med människorna som är inblandade i handeln.

Acknowledgements

I would like to start by thanking **Hugo**, for being a great supervisor! Thank you so much for all your help when Julius and I moved to Sweden and getting us smoothly in the Swedish system, thank you for your patience and support during these years and for always responding extremely quickly no matter what time or where in the world you were. Thank you for teaching me how to write and for introducing me to the world of unlimited stipends for the most amazing travels. It was so much fun being on fieldtrips with you, where you managed to find a perfect balance between work and 'kili-time'. I am amazed by your diplomacy and how calm you are in practically any situation, no matter drivers, 'cheatahs', or other stress-factors. I also want to thank you for always stimulating and supporting me in attending courses and conferences, starting projects and collaborations and for giving me a very large amount of freedom during my PhD. Thank you **Barbara** for your endless enthusiasm for orchids, barcoding, collaborations and interest in applied sciences. It is very inspiring to see how you are able to connect with so many people and make them enthusiastic for your cause. It has been a pleasure to work and travel with you! **Tinde!** You are such an inspiration for me. Thank you for sparking my interest for ethnobotany and tropical plants. Thank you so much for circulating this PhD position among your students! It was the opportunity of a life time and I couldn't be more grateful that I got the change to engage in this project. Also thank you for your support during my PhD, your useful comments and for teaching me how to do market surveys. Mwalimu **Otieno**, asante sana! Thank you for introducing me to Tanzania and for your patience and kindness. It is amazing how you manage to convince everyone to work along with you, from *chikanda* and medicinal plant vendors to people at the ministry. Thank you for teaching me how to perform interviews and thank you for all your guidance during the collecting and traveling. Without you I wouldn't have managed to survive the long bus ride from Dar to Tunduma, or the collecting trip with the piki-piki and the little kano. Thanks **Vincent, Irene, Baset, Anca, Seethapathy, Anneleen, Brecht** from our lab group in Oslo and Uppsala for the interesting get-togethers, discussions, the conferences, courses and the lab retreats.

Thanks to all my current and former colleagues and friends at Systematic Biology! Thank you **Inga** for welcoming me in Uppsala, for giving me and Julius a place to stay, for all the interesting talks about work and cozy dinners and

lunches. You are such an inspiration to me as one of the pioneers in ethnobotany and conservation and I am looking forward to working with you in the future! **Allison,** you made me feel so welcome at Systematic Biology, it was so great to have you as an office mate. Thanks for all the fun, for always taking the time for a talk and to help people, even if you had loads to do yourself. Thanks for the climbing together, for the fun get-togethers you have organized, for… I could go on for a while, but I think you get the idea! Thank you so much **Anders** for all the fun utflykter together with you and Julius, trevliga fika-stunder, running, for teaching me so much about plants, for teaching me Swedish… without you I would have never been able to teach on the floristic course. Thanks for being so patient with me and thanks for helping me move several times and for having me as a neighbor my last months in Uppsala. **Stina**, you are such an inspiration to me! You are by far the kindest and most organized person I know. Thank you so much for all the nice talks and fika-stunder, for the badminton matches, for helping me with teaching so much and for all your support in general! **Sanea**… hehe - awkward smile – thanks so much for everything, all the fun talks, the laughter, the serious talks, thanks for being there for me when times were really tough, thanks for all your funny GIFs, thanks for being you ☺. **Karin!!** You are aweeeesome! Thank you so much for always being so energetic, cheery, friendly, crazy and fun. Thanks for all the fun get-togethers, dinners, badminton matches, cultural outings. **Astrid!** Thanks a lot for joining Julius and me on Valborg boat-watching, fun get-togethers and for those awesome hiking trips in Austria, it has been really great. Thanks **Henrik**, **Martin** and **Mats** for de trevliga fika-stunder and lunches. Thanks for teaching me Swedish, including some old-fashioned words, which I can only use around you… ☺. **Brendan**, thanks for being a great office mate, for all the interesting lunch conversations - I keep being amazed by how you seem to have remembered everything on Wikipedia and more. Thanks also to you and **Milla** for the interesting and fun fieldtrip to Benin – with near-dead experience on the waterfall - and for your cozy Yule-parties. Thanks **Roel** for the interesting conversations, for your amazing Beninese cooking and bringing a bit of Benin to Sweden in the form of baobab-juice and Beninoise. Thanks **Laura** for being such a kind and warm person. Also, thanks for a great time in Benin, it really was a pleasure to do the ethnomycological fieldwork with you ☺. **Julia,** thanks for all of motivational meetings, for the climbing together, for showing me a glimpse of the Stockholm nightlife and for the teaching together on OEM and Floristics. **Petra**, thanks a lot for the nice fika-times, thanks for your support and enthusiasm for teaching and pedagogics and thanks for always having time for me, even when you didn't, it really meant a lot to me. **Micke** thanks for introducing POETS and for your interesting and funny stories. **Agneta**, thank you so much for your colorfulness, for your friendly words and warm smiles and hugs and of course for all your help and support with library-related things! Thank you **Sandie** for your support and for welcoming me into the department. **Raquel**,

thank you for your energy, for all the fun, for your crazy 9GAG posts, cute cat video's, for the after work beers and for keeping me alive with coffee ☺. Thank you **Lore**, awesome officemate and in-house psychologist. Thank you for all the nice conversations, your creativeness, your sparkling energy and for providing me with a daily doses of *Podospora*-soap. **Mahwash** ☺, thanks for always being so kind and cheery and social. **Aaron,** thanks for the climbing, after work beers, pancakes and quizzes ☺. Thanks **Jesper**, for the after work beers, Midsummer parties and quiz-fun. Thanks **Merce** for all the beervolutions, for your energy and for organizing so much fun stuff ☺. Katerina, thanks for your positive energy, for all the deep conversations, thanks for your being so friendly and fun, thanks for saving me with a hamburger on a late lab night and for helping me move when I was so stressed. **Anneli,** thanks for always making me smile! For your kindness, fun fika and for your laughter. **Diem,** you are such an inspiration! Thanks for your energy, for always being so kind and caring, for organizing so much stuff at work and for introducing me to that great dumpling place ☺. **Ioana,** thanks for the talks about Outlander, for your energy and enthusiasm, for your pedagogic skills and your cool karate lesson. **Paco,** thanks for always being so kind and interested. Thanks **Anushree** for your kindness and our fun time in Cesky Krumlov. **Hanna**, thanks for always making me laugh, it is so nice that you joined our department with your group. Thanks to **Sanja** for genuinely caring, I always enjoyed our conversations! **Nahid**! Thank you so much for everything! For your help in the lab, for your kindness and for organizing department fika's and for your interest in others well-being. **Afsaneh,** thank you for your help in the lab and with the teaching-related things and for always making me happy with your friendly smile. Thank you **Karin L.** for being so kind, for your patience with me and for always making time to help even when you had so much to do yourself. Thanks **Iva**, for our nice lunch room conversations and for all of your help! Thanks to **Lillian** for all the help with the teaching and preparing the labs. You are amazing! Thanks to all other people that have been so nice, welcoming and really made me feel at home in Uppsala: **John, Ping, Ding, CJ, Åsa, Stefan, Veera, Markus, Faheema, Fawzeia, Jenni, Sara, Cécile, Fabien, Iker, Jürgen, Caesar, Magnus, David, Thomas, Leif, Stella, Juma, Kristiina, Magda,** all the amazing students that have been at our department over the years, **Mats H.** and **Monika** at the herbarium, **Per, Annika** and **Simon** at the botanical garden.

Thanks to all my collaborators: thank you **Benny** for all your efforts on the identification of our referene vouchers and for working together with us on the *chikanda* projects, thank you **Grace** and **Florent** for sharing your sequences. Thank you **Hildegard** for engaging in our *chikanda* project as well and thank you and **Jean**, and **Flora** and **Leslie**, for having me over in Barrydale for flying and beautiful tours in nature. Thanks to the UPS and MO herbarium for letting me sample collectings for my reference database and thank you **Roy Gereau** for all your kind help at the MO herbarium and making me feel very welcome. Thanks to **Ruth Bone** and **Nicholas Wightman** for getting me involved in the Darwin Initiative chikanda project. It has been great working with you!

Thanks to the funding agencies and stipends who have enable me to do my work: NWO, SIDA, COSTECH, VR, Darwin Initiative, Anna Maria Lundins, Liljewalchs, Helge Ax:son Johnsons Stiftelse.

Thanks to the informants at the markets in Tanzania and Zambia, without whom I would not have been able to do my PhD. A special thanks to **Hashim, Juma** and **Ramadani,** whom have super friendly and helpful.

Thanks to my students who have been so much fun to work with and have been such an inspiration: **Yingzi, Seol-jong, Bert-Jan**. With a special thanks to **Siri, Hanna** and **Chantal** with whom it has been so much fun to do field-work in Tanzania. And **Leah, Samson** and **Zaituni** who have helped us so well during the fieldwork.

A huge thank you to **Rosa** for our fun at conferences, in Paris and New York and for joining me for fieldwork in Tanzania, helping me collecting and interviewing, while experiencing the Dar-es-Salaam heat and market smells and crazy traffic going to Tanga.

A special thanks to everyone who has visited me in Sweden over the years, that really means a lot to me and it has been very nice to have you over **Julius, Egge, Valesca, Marc, Paul, mama, tante Wil, ome Dik, papa, Christa, Martin** en **Mees.** And thanks to all my friends and family for showing interest, for supporting me, for sending me nice emails, cards and packages. It made me feel really warm and happy! I love you all so much!

Last but not least a huge thanks to my family: **Julius**, allerliefste schat! Dank je voor onze heerlijke tijd samen in Zweden en voor alle fijne vakanties in Nederland en Zweden. Je bent het liefste, meest begripvolle, grappige en gezellige kind dat ik me ooit had kunnen wensen. Ik hou zo veel van je! **Mama**… bedankt voor al uw steun, voor alle lieve woorden, lieve kaartjes, voor uw bezoekjes aan Zweden en voor het er altijd voor mij en Julius zijn en het zo fijn te maken om thuis te komen. **Paps** en **Joke**, heel erg bedankt voor al jullie steun, voor de tientalle keren halen en brengen van het vliegveld, voor het skypen, voor de krakelingen. Paps…. zo leuk dat u op bezoek geweest ben in Zweden, ik vond het zo gezellig. En zo leuk dat jullie nu samen komen voor mijn verdediging! **Egge** en **Diane**, heel erg bedankt voor jullie zorgen voor Julius en voor jullie flexibiliteit met betrekking tot de planning. Dat waardeer ik echt enorm.

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