UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 1908

Polyporoid fungi of Tanzania

Taxonomy, transcriptomics and biochemical analyses of Kusaghiporia usambarensis and Piptoporellus baudonii

J. M. HUSSEIN

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020

ISSN 1651-6214
ISBN 978-91-513-0881-4
urn:nbn:se:uu:diva-405548

Dissertation presented at Uppsala University to be publicly examined in Lindahlsalen, Evolutionsbiologiskt centrum (EBC), Norbyvägen 14-18, Uppsala, Friday, 17 April 2020 at 10:00 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Professor Jan Stenlid (Department of Forest Mycology and Plant Pathology; Division of Forest Pathology. Swedish University of Agricultural Sciences).

Abstract
Hussein, J. M. 2020. Polyporoid fungi of Tanzania. Taxonomy, transcriptomics and biochemical analyses of *Kusaghiporia usambarensis* and *Piptoporellus baudonii*. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1908. 56 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0881-4.

Polyporoid fungi refers to basidiomycetes with fruiting bodies with the hymenium located to the inner surfaces of pores or narrow tubes. The majority of polyporoids belongs to *Polyporales.* Most *Polyporales* are saprobes, but some are plant pathogens. The overall aim of this thesis was to study the taxonomy, systematics and chemistry of the two species *Kusaghiporia usambarensis* (saprobic) and *Piptoporellus baudonii* (a plant pathogen) collected from Tanzania, using morphological and molecular approaches, combined with transcriptomics and pharmacognostic investigations.

The main contribution of this thesis includes the description a new genus with the new species *K. usambarensis* from the Usambara Mountains, Tanzania; investigation of the chemical composition of volatile compounds from this medicinal mushroom; isolation and structure determination of a novel and most abundant peptide in *K. usambarensis*, and further to elucidate the phylogenetic position of *Piptoporellus baudonii* (formerly known as *Laetiporus baudonii*) by using a four molecular markers dataset.

Paper **I** was conducted applying a classical taxonomic approach, including both morphological and phylogenetic analyses, to describe a new genus and species *K. usambarensis.* Paper **II,** investigated volatiles and volatile derivatives in dichloromethane extracts of *K. usambarensis* analysed by GC-MS and NMR spectroscopy. The main elements were phenols, and esters, compounds that may explain the formerly reported antioxidant activity and traditional medicinal use of the mushroom. In paper **III,** screening of peptides in *K. usambarensis* revealed a novel cysteine-rich peptide, highly expressed at gene level and the most abundant compound in the fruiting body. Combined LC-MS and transcriptome analyses were used to determine the peptide sequence, and subsequently NMR spectroscopy to determine the 3D structure of the novel peptide, kusaghitide. In paper **IV** molecular techniques were used to elucidate the phylogenetic position of the parasitic *Laetiporus baudonii*. Phylogenetic analyses of combined 5.8S, nrLSU, nrSSU, and TEF1 gene sequences placed *L. baudonii* in the genus *Piptoporellus*, hence the new combination *Piptoporellus baudonii* was proposed. This thesis has contributed to build capacity in the fields of mycology, systematics and pharmacognosy in order to reinforce ecological knowledge and ethnopharmaceutical research for future drug discovery in Tanzania and Africa at large.

Keywords: Systematics, Laetiporaceae, pharmacognosy, cysteine-rich peptide, baudonii

J. M. Hussein, Department of Organismal Biology, Systematic Biology, Norbyv. 18 D, Uppsala University, SE-75236 Uppsala, Sweden.

ISSN 1651-6214
ISBN 978-91-513-0881-4
urn:nbn:se:uu:diva-405548 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-405548)

The future is enthusing

List of Papers

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

I **Hussein, J.M**., Tibuhwa, D.D. and Tibell, S. (2018). Phylogenetic position and taxonomy of *Kusaghiporia usambarensis* gen. et sp. nov. (Polyporales). Mycology, DOI: 10.1080/21501203.2018.1461142.

II **Hussein, J.M**., El-Seedi H., Tibuhwa, D.D., Tibell S., Wedén C. and Göransson, U. (2020). Chemical composition of the edible mushroom *Kusaghiporia usambarensis* (Manuscript).

III **Hussein, J.M**., Tibuhwa, D.D., Wedén, C., Jacobsson, E., Rosengren, K.J., Celestine, C., Tibell, S. and Göransson, U. (2020). Cysteine-rich peptide from gigantic edible mushroom *Kusaghiporia usambarensis* (Laetiporaceae) (Manuscript).

IV Tibuhwa, D.D., **Hussein, J.M.**, Ryvarden, L., Sijaona, M.E.R. and Tibell, S. (2020). Elucidating the phylogeny of the serious plant pathogen *Piptoporellus baudonii* using a multigene molecular dataset (submitted).

Reprints were made with permission from the respective publishers.

I have made primary contribution to the specimen collection, experimental design, data generation and analyses. For **Paper I,** I collected specimens, isolated DNA, performed all analyses, wrote a first draft of the manuscript and contributed to editing of the final version. In **Paper II**, I collected the specimen, performed chemical isolation and GC-MS analyses, prepared the figures and wrote a first draft of the manuscript and contributed to the editing the final version. For **Paper III**, I isolated RNA and peptides, investigated recombinant peptide expression, carried out all the analyses, prepared the figures and wrote a first draft of the manuscript and contributed to editing of the final version. In **Paper IV**, I isolated DNA from a herbarium specimen, performed all the analyses, participated in the writing of the manuscript and contributed to the editing the final version.

Contents

Abbreviations

BLAST	Basic Local Alignment Search Tool
CRP	Cystein rich-peptides
DNA	Deoxyribonucleic acid
DCM	Dichloromethane
HPLC	High performance liquid chromatography
LC	Liquid chromatography
MS	Mass spectrometry
NCBI	National Centre for Biotechnology Information
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
nrLSU	Nuclear Ribosomal Large Subunit
nrSSU	Nuclear Ribosomal Small Subunit
RPB2	RNA Polymerase II second-largest subunit
PTM	Post-translational modification
TEF1	Translation Elongation Factor 1 alpha
TOCSY	Total Correlation Spectroscopy

Introduction

There is lack of capacity in mycology in Tanzania, and also in Africa at large. Due to the lack of capacity in academic mycological expertise, limited records of fungi are available. In Tanzania a rich variation in topography and climatic conditions have offered conditions for a great biodiversity of fungi; nevertheless large areas remain mycologically uncharted and numerous new taxa are yet to be described. Indigenous communities living in forest areas have a rich experience of ethnomycology that still lacks documentation. Sadly, parts of this traditional knowledge and practices are being lost from one generation to another, and the older generation remains the only curators. The projects within my PhD thesis aimed at training me in mycology, in an effort to further build capacity in Tanzania, by advanced training in various fields of mycological research including systematics and biochemical analyses to strengthen ecological knowledge and to document ethnopharmaceutical practices for future drug discovery.

Background of the projects

In my master's project in 2012, I worked on domestication of wild edible mushrooms from Tanzania. Among the study sites where we collected wild edible mushrooms was the indigenous forest of the Usambara Mountains. Prior to going to the forests, we gathered information through questionnaires from indigenous people living near the forests. The gathered information included the type of mushrooms they consumed, how they identified and named them, cooking and preservation methods and any other traditional uses, including myths and medicinal applications.

During our work we collected information on edible mushrooms, but one of these in particular caught our attention. This mushroom, locally named *kusaghizi*, grows by degrading everything that it comes into contact with, including also snakes and insects. The mushroom's fruiting body grows very large and tastes like chicken, and one fruiting body is large enough to be shared among four or five families. Although the *kusaghizi* mushroom is only rarely found it is nevertheless sought after by many people in the area. We were advised to contact the traditional healers 'Elder Andrea' and 'Elder Kijazi'. They told us more about *kusaghizi* and its uses in traditional medicine,

which includes treating diabetes, hypertension, haemorrhoids, and also to increase milk production in lactating mothers. In searching for the mushroom in the forest we found a young *kusaghizi* (Fig. 1A and 1B) weighing about 4 kg. I never before saw a mushroom fruiting body of that size. While I was stunned by the size and weight of the *kusaghizi*, Elder Andrea looked at me and said '*kijana wangu hujaona kitu bado, ukilikuta limekua kabisa utafurahi na roho yako*' - meaning that this was a young mushroom I haven't seen the full-grown *kusaghizi*. We collected the fruiting body for further investigation. Later on, in 2016 when I started my PhD studies, *kusaghizi* was to be part of my study.

I aimed to learn where it belongs in the phylogeny, to screen the chemical compounds from extracts of the fruiting body and to study their biological activity. Because of the unusual growth rate of *kusaghizi*, I wanted to investigate the gene expressions in order to understand its biosynthetic pathways.
To achieve these aims fruiting bodies of *kusaghizi* were collected during the rainy season in February–April 2016, 2017 and 2018. Morphological and DNA barcoding methods were used for characterization; RNA sequencing was used to obtain a transcriptome. For chemical analyses, LC-MS, GC-MS and NMR spectroscopy were used to elucidate the structure of the compounds.

The other part of my thesis is focused on investigating a pathogenic polyporoid fungus that affects a wide range of plants in the southern part of Tanzania (in the Lindi and Mtwara regions). The economy of the area is mostly based on agriculture, and the main crops are *Anacardium occidentale* (cashew nuts), *Cocos nucifera* (coconuts), *Arachis hypogaea* (groundnuts), and *Sesamum indicum* (sesame). Wilting and death of cashew-nut trees, *Manihot esculenta* (cassava) and eucalyptus trees was observed and associated with the attack of a polyporoid fungus resembling *Laetiporus baudonii.* The pathogenic fungus has shown to be very aggressive, and after appearance of the first symptoms of infection, which are loss of deep green color of leaves followed by frequent and rapid wilting of the leaves, the plant dries out and dies within three months. The main objective of this project was to identify and characterize this parasitic polyporoid fungus, for which morphological and DNA barcoding methods were employed.

Figure 1. A) Young fruiting body of *Kusaghiporia usambarensis* (B) Mature fruiting body of *Kusaghiporia usambarensis* after detachment from the tree. From the right: Elder Kijazi (herbalist), Samuel (Kijazi's son), Juma (author) and Elder Mbago (local field guide).

I will hereon introduce to you to the specific features of polyporoid fungi, and then describe the occurrence of interesting chemical substances discovered, mainly phenolic compounds, fatty acids and polypeptides from *Kusaghiporia usambarensis*. This background part will be followed by outlining the aims of the work, and an introduction to the different methods I have used to study *K. usambarensis* and *Piptoporellus baudonii*. Finally I will sum up my main findings from the different studies and how these may be used in future research on the ecology of *K. usambarensis* and also for the pathology of *P. baudonii*.

Polyporoid fungi

Morphology and phylogeny

The term polyporoid fungi or 'poroids' is used to describe fungi having fruit bodies with their hymenium located to the inner surfaces of pores or narrow tubes, and occasionally overfold or shallow depressions between vein-like reticulations (Weiss 1920). Many of the polyporoid fungi belong to *Polyporales*. *Polyporales* is an order of fungi in the phylum Basidiomycota, containing more than 1800 species in 37 families (Kirk et al. 2008; Justo et al. 2017). The majority of the species in *Polyporales* produce fruiting bodies which possess a stalk with a pileus or distinguishable cap with pores or tubes underneath ('polypores'). Some members, however, have gills (*Lentinus*, *Panus*) while others, so called corticoids, produce effused, smooth fruiting bodies (Larsson et al. 2004). The fruiting bodies are annual or perennial and often formed under fallen logs lying on the forest floor, where the microclimates remain wet most of the year. Most *Polyporales* are saprobes, but some are plant pathogens (Banik et al. 2010). Seven clades have been recognised in *Polyporales*: 'antrodia'; 'core polyporoid'; 'residual polyporoid'; 'phlebioid'; 'tyromyces'; 'gelatoporia' and 'fragiliporia' (Fig. 2) (Binder et al. 2013; Zhao et al. 2015).

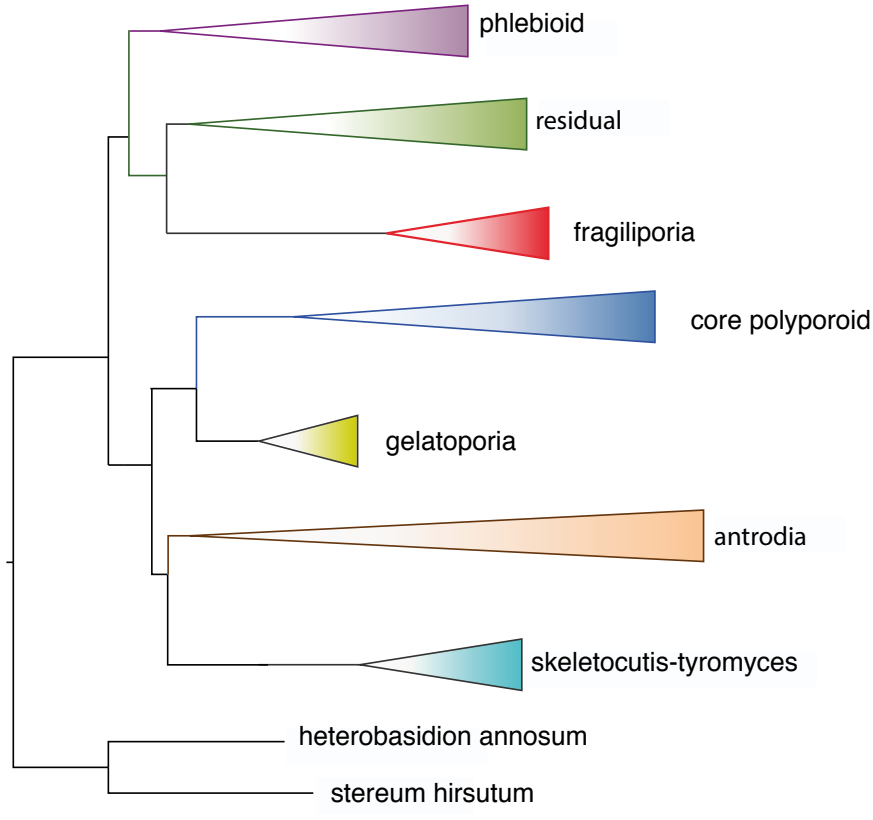

Figure 2. Cladogram depicting the major clades in Polyporales: 'phlebioid'; 'antrodia'; 'core polyporoid'; 'residual polyporoid'; 'skeletocutis-tyromyces'; 'gelatoporia' and 'fragiliporia'. Illustration by author (Hussein et al. 2018).

Importance of polyporoid fungi

Polyporoid fungi are important wood-decaying fungi which contribute to nutrient cycling in forests by decomposing wood debris and thereby contributing to soil improvement by the release of nutrients (Lonsdale et al. 2008). Their fruiting bodies are often collected as food and are included in the local diet of people in various parts of the world and eaten either fresh or dried (Longvah and Deosthale 1998). *Laetiporus sulphureus* and *Kusaghiporia usambarensis*, locally known as *maminu* and *kusaghizi* respectively, are frequently collected in forests of the Usambara Mountains of Tanzania (Powell 2012; Hussein et al. 2018). They are considered as a functional food since they contain a nutritional composition that may help to improve health.

The use of polyporoids as health tonics, tinctures, teas, and food (as soups etc.), as well as in herbal formulas in traditional medicine is common in several communities. Thus e. g. *Wolfiporia cocos* has been used to revitalize the spleen, while *anoderma lucidum* called *reishi* (Chinese) or *mannentake*

(Japanese) has a history of being used in traditional medicine to treat hepatopathy, chronic hepatitis, nephritis, hypertension, arthritis, insomnia, bronchitis, asthma, and gastric ulcers (Kabir et al. 1988). Moreover, extracts from polypores have been shown to modulate the immune system, lower blood pressure and blood lipid concentrations, and inhibit tumors, inflammation, and microbial activities (Davoli et al. 2005). Numerous types of products with potent and unique health improving properties from polypores fruiting bodies and mycelia are available as dietary supplements and consumed in the form of tablets or capsules (Wasser et al. 2000).

Some polypores have also been shown to have a potential in environmentally friendly technologies for bioremediation due to their ability to release extracellular enzymes capable of degrading a wide range of compounds. They have shown astonishing abilities to degrade also recalcitrant pollutants like polycyclic aromatic hydrocarbons (PAHs), oil in polluted soil, mineralisation soils and reduction of heavy metals (Bennet et al. 2002). One example of this is the mycelia of *Trametes versicolor* which have been utilized as a biocatalyst in paper industries to delignify, dechlorinate and decolorize bleached kraft pulp effluent (Adenipekun and Lawal 2012).

Phenolic compounds and unsaturated fatty acids

Phenolic compounds refer to a groups of structurally complex compounds with one or more aromatic ring bearing hydroxyl groups. They range from complex polyphenols such as anthocyanins and flavonoids, to simple phenolic acids and their derivatives. The antioxidant potential of phenolic compounds is due to their ability to neutralize excess radicals of reactive oxygen species in cells (Palacios et al. 2011). Polyphenols have shown immunomodulatory, chemopreventive, bactericidal, anti-inflammatory and anti-fungal activities (Muruke 2014).

Fatty acids have a long carbon-atom backbone ending with a carboxylic group in one end and a methyl group in the other. Most naturally occurring fatty acids have an even number of carbon atoms in their carbon chain and may be either saturated or unsaturated (Fig. 3). Modification of the chain structure results in branched-chain fatty acids, unsaturated fatty acids, and those containing oxygenated groups (Nichols and Sanderson, 2003). Saturated fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0) do not contain any double bonds (Gunstone and Harwood 2007). Unsaturated fatty acids have one or more double bond in the carbon chain. Thus oleic acid (C18:1) has a single double bond at carbon nine (omega-9), linoleic acid (C18:2) two double bonds starting at position 6 (omega-6), and alpha-linolenic acid three double bonds starting at position 3 (omega-3) (Nichols and Sanderson 2003).

Unsaturated fatty acids are known to be abundant in mushrooms (Byrne and Brennan 1975; Barros et al. 2007). They are precursors in reactions leading to the formation of alcohols (1-octen-3-ol, 3-octanol), aldehydes (2-

octenal) and ketones (1-octen-3-one) that contribute to the characteristic flavours of some of the common edible mushrooms (Combet et al. 2006). Linoleic acid, palmitic acid and stearic acid are the most common fatty acids found in mushrooms (Byrne and Brennan 1975). These fatty acids and their esters have been reported to have hypocholesterolemic, hepatoprotective, anti-inflammatory and antimicrobial activities (Krishnamoorthy and Subramaniam 2014).

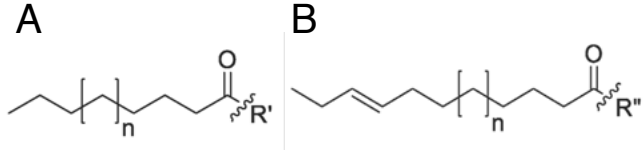

Figure 3. A) saturated and (B) unsaturated skeleton.

Cysteine rich-peptides

Cystein rich-peptides (CRPs) are peptides with a short amino acid chains that contains four to eight cysteine residues which form two to four intramolecular disulfide bridges that cross-link the peptide chain giving them their tertiary structure (Tóth et al. 2016). The disulfide bond increases stability by making the peptide less sensitive to enzymatic degradation, heat degradation and more stable in a broad pH range (Bulaj 2005). CRPs are produced by all groups of organisms. They show potent antimicrobial activities against human, plant, and animal pathogens, as well as food-borne pathogens (Marx 2004). They are categorized in classes based on their portrayed activities such as ribotoxins, which inactivate the ribosomes by breaking a single phosphodiester bond of the rRNA (Olombrada et al. 2017), or protease inhibitors (Eguchi et al. 1994). Apart from protection against pathogens, CPRs have also shown uterotonic properties, as for instance kalata B1 from the plant *Oldenlandia affinis*. An example of this effect as being practiced in traditional medicine, is found in the Democratic Republic of Congo where pregnant women would drink a concoction of *O. affinis* leaves to accelerate labor (Sletten and Gran 1973).

Aims

The overall aim of the projects included in this thesis was to investigate the polyporoid fungi *K. usambarensis and P. baudonii* from Tanzania by using morphological and molecular approaches, combined with a pharmacognostic approach.

The specific aims of my doctorate were:

- To describe a basidiomycetous fungus new to science collected from Tanzania, **paper I.**
- To investigate whether this fungus, which has been used in traditional medicine, contains bioactive compounds, **paper II.**
- To understand the connection between the expression at gene level and an abundant peptide isolated from *kusaghizi*, and elucidating the peptide's chemical structure, **paper III.**
- To reveal the phylogenetic position of a basidiomycete that is a serious crop pathogen by using molecular data, **paper IV.**

Interdisciplinary approach

Papers **I, II and III** comprise an interdisciplinary approach to study *K. usambarensis* (Fig. 4). For paper **IV** a systematic approach was used. For systematics studies the fruiting bodies of both *K. usambarensis and P. baudonii* were collected from the forests, characterized by morphological features followed by phylogenetic analyses using multi-gene datasets. To investigate the chemical constituents of *K. usambarensis*, dichloromethane was used for isolation, followed by fractionation on columns packed with silica then analysed by nuclear magnetic resonance (NMR) spectroscopy and gas chromatography mass spectrometry (GC-MS). In transcriptomic analysis, RNA were isolated from a fruiting body preserved in RNAlater ®, RNA-seq generated by illumina technology and a *de novo* assembly using the Trinity software. Screening of the peptides was achieved by purification of 50% aqueous methanol extract by fast protein liquid chromatography (FPLC). Peptide sequencing was accomplished by liquid chromatography mass spectrometry, and the transcriptome from RNA-seq was used as database to search for the complete gene of the peptides. Finally, the structure of peptides was elucidated by NMR spectroscopy using isotopic labelled peptide with $^{15}N/^{13}C$ produced by recombinant expression on *Escherichia coli*. The results from phylogenetic analyses provided additional information on the studied peptide.

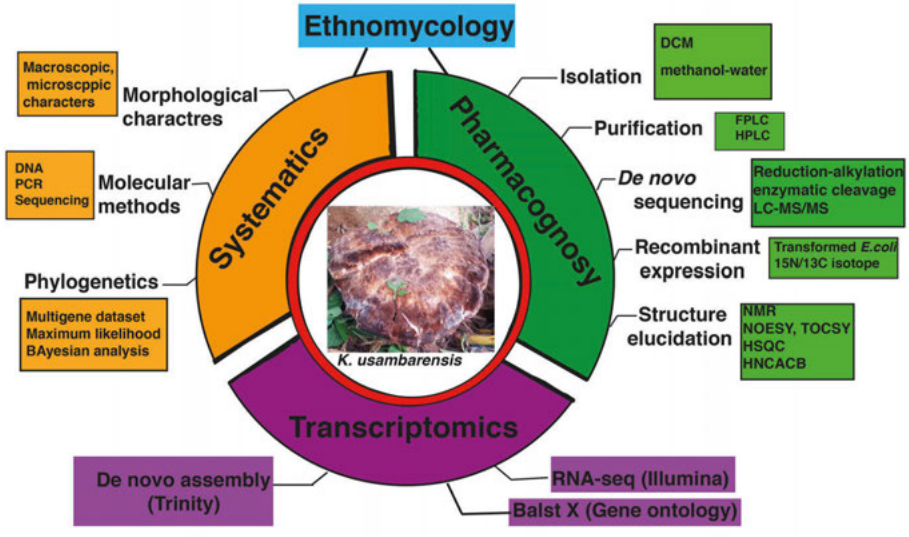

Figure 4. An illustration of the interdisciplinary approach applied in this thesis. Illustration by author.

Study sites

West Usambara Mountains, Tanga

The Eastern Arc Mountains of Tanzania are a series of isolated crystalline Precambrian basement mountains stretching from the northeast (Pare) to the south-central (Udzungwa) in Tanzania (Fig. 5A; Hall et al. 2009). These fault-block mountains were formed about 30 million years ago.

Field work for the first project was conducted at villages in the West Usambara Mountains located in the Korogwe District of the Tanga region in the North Eastern part of Tanzania. Material of the fungus under study was collected during the rainy seasons in 2016-2018.

Forests of the Eastern Arc mountain blocks are sequestered from each other and famous for their high species richness and endemism (Lovett 1990; Burgess et al. 2007). Biologists regard them as an exceptional ecosystem among global biodiversity hotspots due to the high richness of rare and endemic species. These mountainous forests have a constant cloud mist and frequent precipitation due to the high elevation and to the wind effects from the Indian Ocean, which support growth and diversity (Myers et al. 2000). The Usambara Mountains are inhabited by the Sambaa, Bondei, and Zigua ethnic groups. People dwelling around the Usambara Mountains are an important source of indigenous knowledge on edibility and other uses of the studied mushrooms.

Southern regions, Lindi and Mtwara

Lindi and Mtwara are located in the South-eastern part of Tanzania with its main socio-economic activity being agriculture. The economy of people in these regions greatly depends on cash crops such as cashew nuts, groundnuts, sesame and coconuts. The farmers' prosperity and respect appear to be determined by the number of cashew nut trees possessed, which contribute to high revenue during the harvest season (www.jica.go).

The field work for paper **IV** on *Piptoporellus baudonii* was conducted in the Mtwara and Lindi regions (Fig. 5 B) in the southern parts of Tanzania, where cashew nuts is a major cash crop that recently often has been affected by this fungus. These regions have a high mean relative humidity of up to 87% in February – June, which corresponds to the long rain season, while the lowest relative humidity of 63% occurs in September – October. Temperatures vary with cold months (June – September) having a mean temperature of 19.5ºC, while the hot months (September – December) have ca. 30ºC. The soils are sandy loam or red clay soils, receiving an annual rainfall from 900 to 1200 mm (Sijaona and Shija 2005).

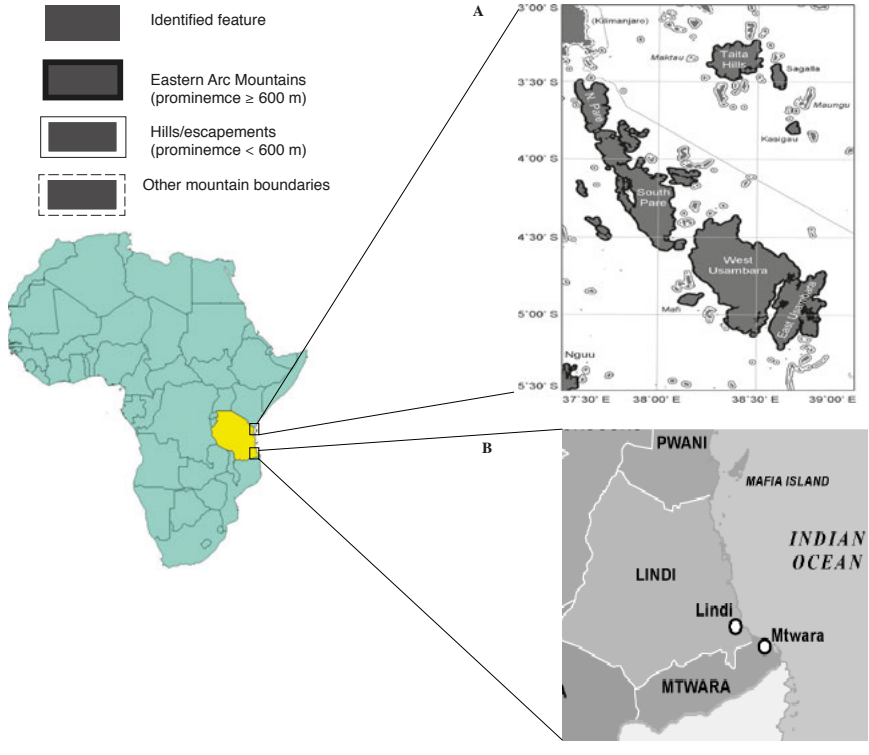

Figure 5. (A) Map showing the Pare and the Usambara blocks of the Eastern arch mountains. The appeared block including: Taita hills, Northa Pare, South Pare, West Usambara, East Usambara and Nguu. After Platts et al. 2011. (B) The Lindi and Mtwara regions (EMapsworld).

Morphological classification

The polyporoid fungus *kusaghizi* was in this thesis described scientifically i. a. based on its morphological characters. Morphological characters used in describing this species were cap size, colour, consistency of the cap, stalk attachment and structure, mode of gill attachment to the stalk, spore colour in mass, bruising and chemical reactions (Arora and Hershey 1986). Microscopical features studied on the fresh and dry fruiting bodies included hyphal system, cystidia, basidia, and basidiospore morphology. These features were also used for comparison with other polyporoid fungi (Bougher and Syme 1998).

Molecular phylogeny

Taxonomic and phylogenetic studies of basidiomycetes by comparing morphological characters have left many questions and controversies unresolved, in part due to the scarcity and plasticity of characters of fungal basidiocarps (Hibbett 2007). Nucleic acid technology has facilitated a rapid and more robust classification of fungi by utilizing DNA sequence information, and has by large supported earlier morphology-based classification systems (Razaq et al. 2013).

The application of multi-locus analyses has provided robust and detailed *Polyporales* phylogenies. Multi-locus analyses combining rDNA (ITS, nrLSU and nrSSU) with protein-coding genes, particularly RNA polymerase II subunits two (RPB2) and Translation elongation factor 1-alpha (TEF1), have been used to create a comprehensive dataset for inferring phylogenies (Hibbett 2007; Zhao et al. 2015). Efforts to use more genes for phylogenetic inference has also been attempted by using whole genome analysis. Binder et al. (2013) used a phylogenomic dataset (2,571,356 genes) from 3 different *Polyporales* species in comparison with a comprehensive six-gene dataset (5.8S, nrLSU, nrSSU, RPB1, RPB2, TEF1) of 373 taxa in *Polyporales*.

To correctly describe and identify *kusaghizi*, DNA was isolated, amplified, sequenced and analysed. This was the basis for to providing additional information of the evolutionary relationships within the *Polyporales*. The genes nrLSU, nrSSU, RPB2 and TEF1 were amplified, sequenced and used to infer phylogenetic relationships. These genes were analysed individually, before they were concatenated, in order to ascertain that there was no significant incongruence among the single-gene trees.

GC-MS and analyses of phenolic and unsaturated fatty acids

Gas chromatography in combination with mass spectrometry (GC-MS) is a powerful analytical method for analyzing volatiles and volatile derivatives. It combines features from gas chromatography and mass spectrometry to detect

volatile constituents in the sample (Kitson et al. 1996). For non-volatile compounds, silylation is performed to enhance compound volatility in order to be perform GC-MS analyses. Usually, the substrate is deprotonated with an appropriate strong base prior to the treatment with a silyl chloride (Blau & Halket 1993). A mixture of compounds extracted by solid phase microextraction (SPME), or by solvents such as dichloromethane, can be analyzed by GC-MS (Risticevic et al. 2009). Both retention indices and mass spectra from the analysis are compared to a spectrum library search for matches.

For the analysis performed in this thesis, compounds were extracted from the fruiting body by DCM and silylated by bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) prior to the analysis by GC-MS. Compounds were tentatively identified using the NIST 2008 MS Library employing the retention indices and the MS spectra.

RNA sequencing

RNA sequencing and analysis

In recent years, advances in technology have made DNA sequencing more cost-effective, faster and enabled sequencing of cDNA resulting from cellular RNA by massively parallel sequencing technologies referred to as RNA-seq (Mortazavi et al. 2008; Garber et al. 2011). RNA-seq has provided a novel opportunity to detect the level of gene expression and novel transcripts (Wu et al. 2014). It is more efficient, sensitive, and assist faster gene discovery and accurate profiles of the transcriptome as compared to microarray analysis or other earlier techniques (Feng et al. 2012). RNA-seq generates short reads (36–125 bases) that are aligned to either a reference transcriptome or genome, if available (Wu and Watanabe 2005). In the absence of a reference genome or transcriptome, *de novo* transcriptome assembly is feasible with, e. g., the Trinity software (Grabherr et al. 2011).

To obtain the transcriptome used in this thesis, total RNA was isolated from fruiting body pieces preserved in RNA later®. After RNA integrity was assessed using a bioanalyzer, RNA-seq were generated using illumina sequencing technology (Bentley et al. 2008) at Macrogen Inc. Due to the absence of a reference genome, the reads were assembled using the Trinity software (Grabherr et al. 2011).

Isolation and analyses of peptides

Peptide extraction and purification

Mushroom fruit bodies contain a mixture of compounds that may be extracted and then purified in order to isolate the compounds of interest. Solvents are

used to extraction of compounds based on their solubility. Non-polar solvents such as dichloromethane, hexane and petroleum ether will dissolve non-polar compounds whereas polar solvents like water, methanol and acetonitrile tend to dissolve polar and semi-polar compounds (Salem et al. 2016). Subsequent purification is achieved by separation methods such as fast protein liquid chromatography (FPLC) and high performance liquid chromatography (HPLC) (Tangvarasittichai et al. 2009).

The peptides isolated in this work were isolated using a methanol-water solution 1:1. Further purification was performed by FPLC, under the gradient ranged from 5 to 40% acetonitrile (AcN) over 60 minutes using a flow rate of 10 ml/min. The eluent was continuously monitored at wavelengths of 254 nm, and fractions were collected automatically.

Identification, reduction, alkylation and enzymatic cleavage of peptides

For the identification of cysteine-rich peptides the disulfide bonds are reduced and alkylated, which leads to a destabilization of the tertiary structure and unfolding of the peptide for better enzymatic activity (Hale et al. 2004). Unfolded peptides are cleaved by either trypsin, endoproteinase GluC or chymotrypsin. These enzymes are specific; trypsin specifically cuts the peptide on the carboxy-terminal side of lysine and arginine; GluC cuts after glutamic acid and chymotrypsin cleaves after tryptophan and tyrosine residues (Steen and Mann 2004).

The reduction process disrupts the disulfide bond, while alkylation prevents its re-formation. Additional information about the number of cysteine residues can be obtained from the procedure, since alkylation by iodoacetamide causes an increment of 58 Da per cysteine residue involved in a disulfide bond (Henschen 1986). For the work in this thesis the peptides were reduced by dithiothreitol (DTT) and alkylated using iodoacetamide (IAM) prior to enzymatic cleavage with trypsin.

De-novo peptide sequencing based on Mass Spectrometry

Advances in liquid chromatography–mass spectrometry (LC–MS) methods have been very efficient in peptide analysis (Chen et al. 2018). LC separates different chemical components as they move at different speed through the column due to varying affinities to the stationary phase in relation to the mobile phase (the solvent passing through the column) (Hamilton and Sewell 1982). The tryptic peptides are eluted from the column using a solvent gradient of decreasing polarity, so that they elute in the order of their polarity. More polar peptides bind weakly to the column and elute fast, while less polar peptides bind strongly, hence elute last (Steen and Mann 2004). Subsequently,

separated tryptic peptides reach at the end of the column and flows through the needle tip. The liquid is vaporized, then peptides are ionized due to strong electric potential (electrospray ionization) producing precursor ions (Fenn et al. 1989).

The precursor ions are imparted by energy collisions with a noble gas (N_2, Ar or He) that fragment it further to product ions. A mass spectrum of these subsequent fragments is produced by the tandem MS also known as a MS/MS spectrum (Arnott 2001.). In the collision induced fragmentation of the peptide, cleavage commonly happens at amide bonds and b-ions, y-ions and rarely a-ions are produced. (Fig. 6). The amino acid sequence is inferred by the mass difference between neighboring peaks in a series. However, near-isobaric amino acid (glutamine/lysine) and isobaric amino acids (leucine/isoleucine) are difficult to differentiate due to their identical masses, hence causing a limitation of the process (Papayannopoulos 1995). The challenge may be overcome by taking advantage of availability of genetic data such as transcriptomes.

For the work reported in this thesis, the digested peptide was lyophilized to dryness in SpeedVac (instrument details), resuspended in AcN 5% in 0.1% TFA then analysed using a UPLC-QToF nanospray MS (Waters nanoAcquity, QToF Micro; 75 µm × 250 mm 1.7 µm BEH130 C18). Scan window was set to 300-2000 m/z and for MSMS to 50-2000 m/z.

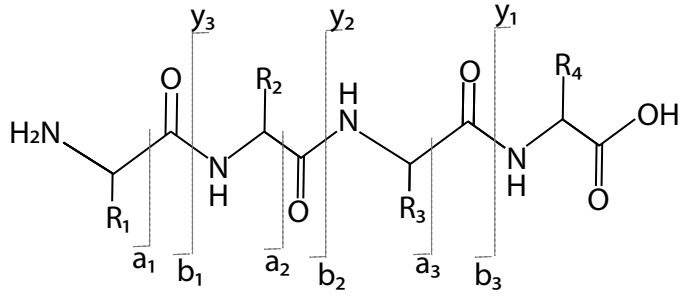

Figure 6. Fragmentation of peptides with y, b and a ion-types presented. R 1-4; side chains for the amino acids 1-4.

Transcriptome-assisted sequencing of peptide

To obtain the full sequence of the peptide only by depending on mass tandem *de novo* sequencing is labour intensive and its success is not guaranteed. This is because some proteins contain isobaric amino acids, do not ionize properly under MS/MS or contain N-terminally blocked residues (Wellner et al. 1990). Transcriptome guided sequencing can be exploited to overcome these challenges to produce the full peptide sequence. Having a short stretch of sequence from *de novo* sequencing by MS/MS experiments, a full sequence

can be obtained by searching in the transcriptome. A programme such as fuzzpro/trans (Rice et al. 2000) is used to search for the regular expression base approach to get the exact sequence match. The translation of nucleotides into protein sequences to all possible six reading frames, is a crucial step in searching for regular expression of the transcriptome. Resulting sequences are assessed to identify mature peptide, pro region. The full peptide sequence can be used as a query to search in other open access databases using a basic local search tool (BLAST) to locate homologous sequences.

The transcriptome used in this thesis, originated from RNA isolated from fruit body sequenced by the illumina sequencing technology (Bentley et al. 2008) at Macrogen, thereafter assembled using the Trinity software (Grabherr et al. 2011). Short sequence fragments of amino acids obtained were explored in translated transcriptome by Regular expression searches to obtain the full-length gene with precursor.

Recombinant peptide expression and laboratory scale production

Recombinant expression of peptides empowers us to produce sufficient amounts for structure determination by NMR spectroscopy (Geron 2020). Producing peptides with isotopic labelled (^{15}N and ^{13}C) offers additional advantages in multidimensional, heteronuclear NMR studies (Kwan et al., 2011). *Escherichia coli* has been the host of choice for recombinant peptide expression due to its fast growth that enable them to reach the required population size (OD_{600} of 0.8–1.0) for peptides expression quicker. Transforming *E. coli* with a plasmid is easy and fast, and their growth media may be produced from inexpensive and easily available materials (Pope and Kenth 1996).

Vector design is crucial for successful peptide expression. Commercially accessible plasmids, like the pET-22b(+) vector (GenScript) that in addition to the peptide sequence of interest also contains N-terminal His-tag for affinity purification, The Tobacco Etch Virus protease (TEV) cleavage site and the ampicillin resistance gene to ensure that only transformed *E.coli* will grow in the media. Transformed *E. coli* can be grown on the Luria-Bertani medium (LB) and minimal medium (M9) for ^{15}N and ^{13}C-labeling; expression of the peptide is induced by β-D-1-thiogalactopyranoside (IPTG). Expressed peptides are recovered by sonication to disrupt the cells followed by purification by immobilized metal affinity chromatography (IMAC) where the His-tag bind to resins. Purified peptides are digested with TEV to separate peptides from additional tags prior purification by FPLC and checked with Matrix Assisted Laser Desorption Ionization time of flight mass spectrometry (MALDI-TOF). Peptides concentrations are frequently being assessed using absorbance

A_{280} in NanoDrop (ThermoScientific) (Gaberc-Porekar and Menart 2001; Karlsson et al. 2015).

For the recombinant expression in this thesis, the One Shot BL21 Star *E.coli* strain (Thermo Fischer Scientific) was transformed by the pET-22b (+) vector (GenScript). Expression was induced by IPTG, protein was purified by Sepharose (GE Healthcare), followed by further purification with FPLC after cleavage and mass was ascertained in MALDI-TOF.

Peptide structural determination using Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy has proven to be a powerful technique for studying peptides structures and dynamics (Kanelis et al. 2001). Each amino acid residue in a peptide has its own spin system that produces an unique pattern of cross peaks. By applying Total correlation spectroscopy (TOCSY) that links amide protons with its alpha proton (Hα) and side chain protons enable the identification individual amino acid spin system. The nuclear Overhauser effect spectroscopy (NOESY) is another important experiment that provides information about consecutive connectivity and inter-proton distances between adjacent amino acid residues (Schroeder and Rosengren 2020).

Among the challenges for structural elucidation of larger peptide molecules, is the presence of more than 500 protons per molecule. As results more than one amide proton may share the same chemical shift which leads to overlap of resonance lines that complicates data analysis. To overcome this challenge, spectra are extended into a cube to generate a multi-dimensional data set. This is achieved by the labeling of the peptide with ^{15}N and ^{13}C and recording both experiments that correlate information between ^{15}N, ^{13}C, and ^{1}H spins at the same time. Experiments such as the correlation of HNCαCβ (^{1}HN) amide proton and ^{15}N chemical shift of residue *i* with the $^{13}C\alpha$ and $^{13}C\beta$ shifts of residues previous residue *i-1* and *i*, while (Hβ)CβCα(CO)NNH experiment correlate ^{1}HN and ^{15}N of residue with $^{13}C\alpha$ and $^{13}C\beta$ shifts of the preceding residue *i-1* only. Thus differentiating between intra- and inter residue correlations in the HNCαCβ (Kanelis et al. 200). After assigning individual spin systems, residues can then be linked in a sequential way along the peptide chain 'sequential walk' using the NOESY spectra (Wüthrich 1986).

In this thesis, NMR spectroscopy was initially analyzed by using TOCSY and NOESY. Since the peptide contained 54 amino acid residues, this resulted in peak overlaps which were difficult to assign. Using peptide labeled with ^{15}N and ^{13}C additional experiments like HNCαCβ, HNCα, HNCαCO and (Hβ)CβCα(CO)NNH were used to ascertain the assignment.

Results and discussion

Summary of Paper I: Description and phylogenetic position of *Kusaghiporia usambarensis*

Hussein, J.M., Tibuhwa, D.D. and Tibell, S. (2018). Phylogenetic position and taxonomy of *Kusaghiporia usambarensis* gen. et sp. nov. (Polyporales). *Mycology* 9, 136–144.

Kusaghizi is a large polyporoid mushroom from the West Usambara Mountains in North-eastern Tanzania. The name is native to the Sambaa tribe, one among the three ethnic groups in the Usambara Mountains, and describe its growth characteristics of devouring anything comes into contact with the fruiting body. At maturity, the large, dark brown fruiting bodies measure up to 60 cm in diameter and may weigh more than 10 kg (Fig. 7). It has a high rate of mycelial growth and regeneration and was found growing on both dry and green leaves of shrubs; attached to the base of living trees, and it was occasionally observed to degrade even dead snakes and insects which had come into contact with it.

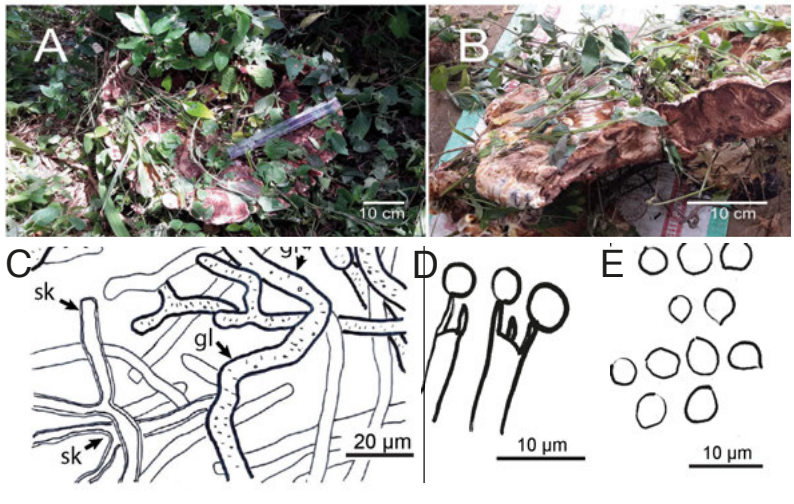

Figure 7. (A) Basidiocarp of *Kusaghiporia usambarensis* (holotype). (B) Vertical section of basidiocarp. (C) Skeletal hyphae (sk) with Y-shaped branches; gloeplerous hyphae (gl). (D) Basidia with spores attached to sterigmata. (E) Globular to subglobular basidiospores.

In this paper, the phylogenetic position of the *kusaghizi* was investigated based on individual and concatenated data sets of nrLSU, nrSSU, and the RPB2 and TEF1 genes. Analyses were based on a total of 209 sequences representing 201 species of *Polyporales*, with two *russuloid* species as out-group. The analyses found *Kusaghiporia* to be nested within the 'antrodia clade' in all analyses (Fig. 8). *Kusaghiporia usambarensis* is morphologically similar to *Phaeolus, Wolfiporia and Laetiporus,* insofar that they all have simple septate hyphae, produce annual polyporoid fruiting bodies with hyaline spores and cause brown rots.

Phaeolus differs from *K. usambarensis* in having a monomitic hyphal system and producing hymenial cystidia (Lindner and Banik 2008). *Wolfiporia* and *Laetiporus*, like *K. usambarensis*, have dimitic hyphal systems. *Wolfiporia*, however, has resupinate basidiocarps and oblong-ellipsoid basidiospores (Zmitrovich et al. 2006). With the exception of *L. persicinus,* other *Laetiporus* species produce brightly coloured basidiocarps (Lindner and Banik 2008). *Kusaghiporia usambarensis* is different from *L. persicinus* in basidioma morphology (up to 60 cm) and the basiodiospores being globose to subglobose, while broadly-ovoid in *L. persicinus*. Despite the morphological resemblance between *K. usambarensis* and *L. persicinus* they are not genetically closely related. Based on its morphological features and molecular data, *kusaghizi* is here scientifically described as *Kusaghiporia usambarensis*. The generic name honours the local *Sambaa* name *kusaghizi* and the species epithet the place where the mushroom was foung, the Usambara Mountains.

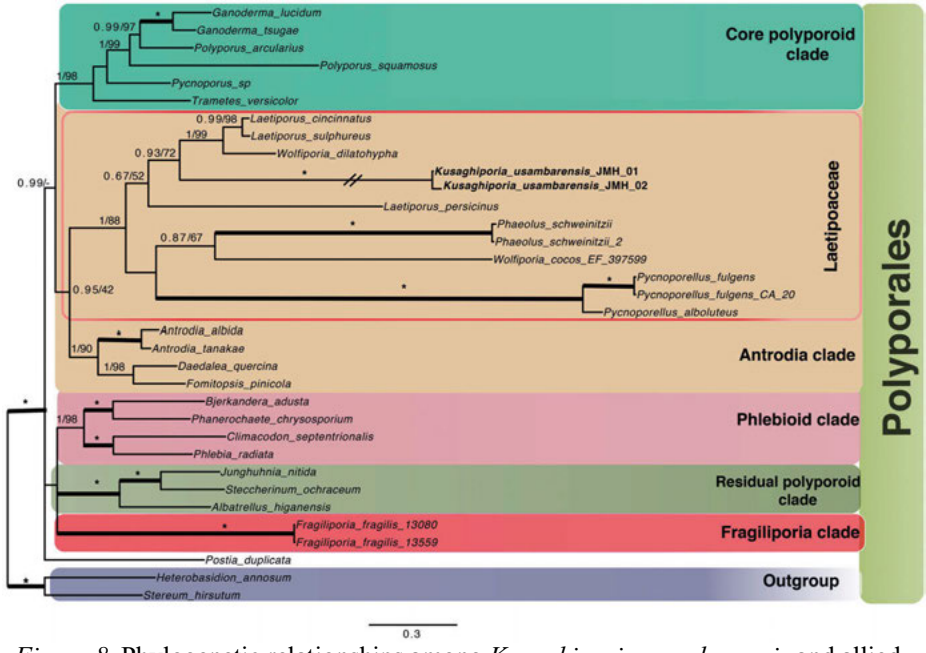

Figure 8. Phylogenetic relationships among *Kusaghiporia usambarensis* and allied taxa in Polyporales, based on Bayesian and ML analyses of concatenated nrLSU, nrSSU, RPB1 and TEF1 datasets. The tree was rooted using two species from Russulales (*Heterobasidion annosum* and *Stereum hirsutum*). The two support values associated with each internal branch correspond to PPs and MLbs proportions, respectively. Branches in bold indicate a support of PP ≥ 0.95 and MLbs ≥ 70%. An asterisk on a bold branch indicates that this node has a support of PP = 1.0 and MLbs = 100. The branch with double-slash is shortened. Clade names follow Zhao et al. (2015).

Summary of Paper II: Fatty acids and volatiles from *K. usambarensis*

Hussein, J.M., El-Seedi H., Tibuhwa, D.D., Tibell, S., Wedén, C., and Göransson, U. (2020). Chemical composition of the edible mushroom *Kusaghiporia usambarensis* (Manuscript).

Kusaghiporia usambarensis is used as food and also in traditional medicine. As food, it is highly esteemed by the local community due to its delicious chicken like taste (personal observation), and the very large fruiting bodies may be shared between three to four families. In traditional medicine, it has been used to lower blood sugar and high blood pressure. The fruiting body is for medicinal purposes dried, ground to a powder and finally mixed with warm water to produce a concoction or porridge (Hussein et al. 2018). It has also been used for the treatment of haemorrhoids and to increase milk production in lactating mothers. *Kusaghiporia usambarensis* was only quite recently described as a new genus and species, and its chemical composition has so far not been investigated.

In order to gain insight into both the basic biology and the traditional use of *Kusaghiporia usambarensis*, a dichloromethane (DCM) extract of a dried fruiting body was analysed by nuclear magnetic resonance and gas chromatography coupled to mass spectrometry. The DCM extract was fractionated by column chromatography packed with silica. The fractions on NMR showed the presence of long chain fatty acids (Fig. 9A). Gas chromatography mass spectrometry can only detect volatile compounds and silylation was therefore necessary to enable detection of the non-volatiles as well.

A total of one hundred and ten (110) compounds were tentatively identified using the NIST 2008 MS Library employing the retention indices and the MS spectra. Identified compounds were grouped in classes; esters, hydrocarbons, oxygenated hydrocarbons, heterocyclic, aromatic hydrocarbons, fatty acids and steroids. The main constituents were 2,4-bis(1,1-dimethylethyl) phenol (substance 90) and (91) Trimethyl(2,6 ditert.-butylphenoxy)silane, which accounts for about 22% of the extract (Fig. 9B). Phenolic compounds from *K. usambarensis* have been shown to have the ability to scavenge free radicals and reactive oxygen species (ROS) and may have health effects (Juma et al. 2016). Phenolic compounds in mushrooms have been associated with defense mechanism against microorganisms and insects (Petrova et al. 2007). They have also shown antitumor, antioxidant, anti-androgenic, antihistaminic, antimicrobial and anti-inflammatory activities (Adeoye-Isijola et al. 2018).

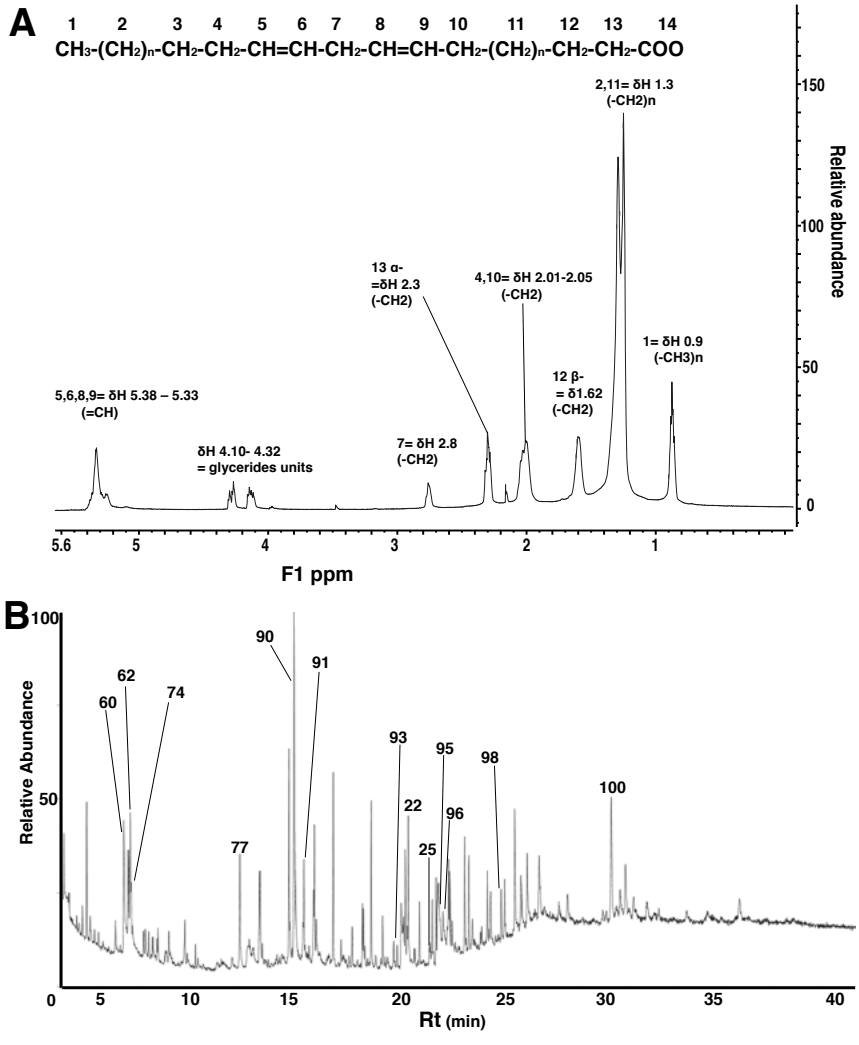

Figure 9. (A) NMR chromatogram one of the oily fraction, proton 1H NMR and a skeleton of long-chain fatty acid (B). GC–MS chromatogram of *Kusaghiporia usambarensis*. Annotated peak numbers correspond to constituents listed in Table 1. 22-palmitic acid, ethyl ester, 25-heptadecanoic acid, ethyl ester, 60-1-octen-3-one, 62-hexanoic acid (caproic acid), 63-octanal, 74- 2-pentylfuran, 77-furan, 2-hexyl-, 90-phenol, 2,4-bis(1,1-dimethylethyl)-, 91-trimethyl(2,6 ditert.-butylphenoxy)silane, 93-n-hexadecanoic acid (palmitic acid), 95- 9,12-octadecadienoic acid (Z,Z)- (linoleic acid), 98-androstane, (5β)-, 100-anthraergostatetraenol benzoate,

Volatile compounds contribute to the aroma and flavour of the mushrooms. Thus 1-octen-3-one (60) (Fig 9B) has a mushroom-like smell with nuances of cabbage and broccoli in addition to minor savory notes of fish and chicken (Mosciano 1993). Octanal (63) has a citrus-orange with green peel and chicken like nuance (Mosciano 1993). 3-octen-2-one (65) has a sweet,

blueberry, and crushed bug taste (Arn and Acree 1998). A combination of these volatiles contributes to the mushroomy taste. In addition to flavour, octanal and nonanal have been shown to have antifungal activity (Tao et al. 2014).

Hexanoic acid (62), has been reported to show antimicrobial activity (Huang et al. 2011). Likewise heptadecanoic ethyl ester (25) possesses antimicrobial and antioxidant activity (Suseem and Saral 2013). Octadecanoic acid (95) (Figure 9B) has numerous biological activities, such as hypocholesterolemic, anti-inflammatory and anti-arthritis, antieczemic, anticoronary, hepatoprotective, antimicrobial and anticancer (Krishnamoorthy and Subramaniam 2014; Yu et al. 2005). Palmitic acid (93) and palmitic acid ester (22) are known for their hypocholesterolemic effect (Adeoye-Isijola et al. 2018; Yu et al. 2005). High contents of phenols and esters compounds may explain the earlier reported antioxidant activity and traditional medicinal use of the mushroom.

The chemical analysis has also shed light on *K. usambarensis* as a preferred delicacy: the presence of 1-octen-3-one, octanal, 3-octen-2-one contribute to its fruity, cheesy and chicken-like flavour.

Summary of Paper III: Cysteine-rich peptide from *K. usambarensis*

Hussein, J.M., Tibuhwa, D.D., Wedén, C., Jacobsson, E., Rosengren, K.J., Celestine, C., Tibell, S. and Göransson, U. (2020). Cysteine-rich peptide from gigantic edible mushroom *Kusaghiporia usambarensis* (Laetiporaceae) (Manuscript).

To obtain an overview of the chemistry of *Kusaghiporia usambarensis* we investigated different types of extracts. In paper III we focused on aqueous methanol extracts and employed a multidisciplinary approach to understand the connection between the expression at gene level and the final product of the expression (a peptide), and to elucidate the chemical structure of the expressed peptide. To achieve this we isolated and sequenced RNA from *K. usambarensis* to generate a transcriptome that was used as a database in connection with the data produced by the LC-MS/MS analysis for studying the isolated peptide. To elucidate the structure of this peptide, it was expressed in a transformed *E.coli.* The expressed peptide was labeled with ^{15}N and ^{13}C isotopes and analyzed by NMR spectroscopy.

RNA sequencing was done using Illumina HiSeq and assembled by the Trinity software (Grabherr et al. 2011). From five replicates, a total length of 187,485,055 bp, 74,339 contigs were revealed by *de novo* assembly transcripts and a total of 23,599 genes were annotated and subjected to Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

From the frozen fruiting body, 2.5 kg was dried overnight at 38 °C, pulverized to a fine powder (600 g) and used for sequential extraction with dichloromethane (DCM); methanol; and finally methanol: water (1:1). The methanol: water fraction produced 12.5 g of a semisolid extract. One g of this was fractionated in FPLC to obtain the pure peptide. The purity of the peptide was assessed on HPLC (Fig. 10A). LC-MS analysis of the peptide showed peaks with a mass-to-charge (m/z) ratio ($M+H^+$) of 5612.02 (mo.) (Fig. 10A)

Results from the reduction and alkylation of the purified peptide showed an increase in total mass with a mass-to-charge of 5960.54 (mo.) which demonstrated that the peptide contains six cysteine residues corresponding to three disulfide bridges (Fig. 10B). The alkylated peptide was enzymatically cleaved to produce fragments for *de novo* sequencing using data from LC-MSMS. Short sequence fragments of the seven amino acids (SYTGYDC) obtained were explored in a translated transcriptome by Regular expression searches which resulted in a full-length gene with a precursor of 39 amino acids, and a 54 aminoacid mature peptide (kusaghitide) (Fig. 11 A-C). A Regular expression search of the translated transcriptome revealed six identical sequences expressed from six isoform genes. Isoforms are thought to improve the endurance of these molecules to physical and chemical stresses and to increase chances for their production (Butala et al. 2015). The experimental

mass of the peptide differed from the predicted mass calculated from the complete amino acid sequence from transcriptome analyses by -17 Da. The deficit in mass suggests a posttranslational modification of the N-terminal first amino acid Glutamine (Q) to Pyroglutamic acid (https://abrf.org/delta-mass).

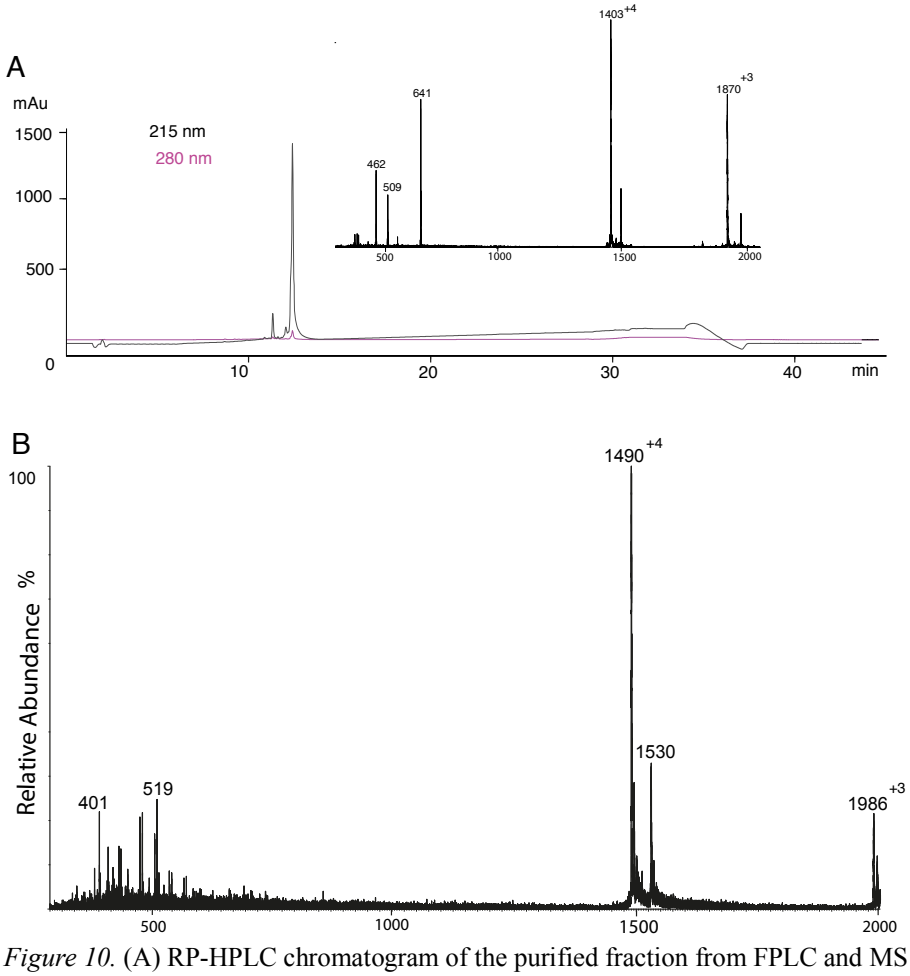

Figure 10. (A) RP-HPLC chromatogram of the purified fraction from FPLC and MS spectra of the purified peptide before reduction and alkylation. The peaks with mass to charge ration (m/z) of 1403 and 1870 corresponds to four and three charges respectively that gives ($M+H^+$) of 5612.02 (mo.). (B) MS spectra of reduced and alkylated peptide using iodoacetamide (Cys + 57). The peaks with mass to charge ration (m/z) of 1490 and 1986 corresponds to four and three charges respectively that gives ($M+H^+$) of 5960.54 (mo.). This increase in total mass demonstrates that the peptide contains six cysteine residues that correspond to three disulfide bridges.

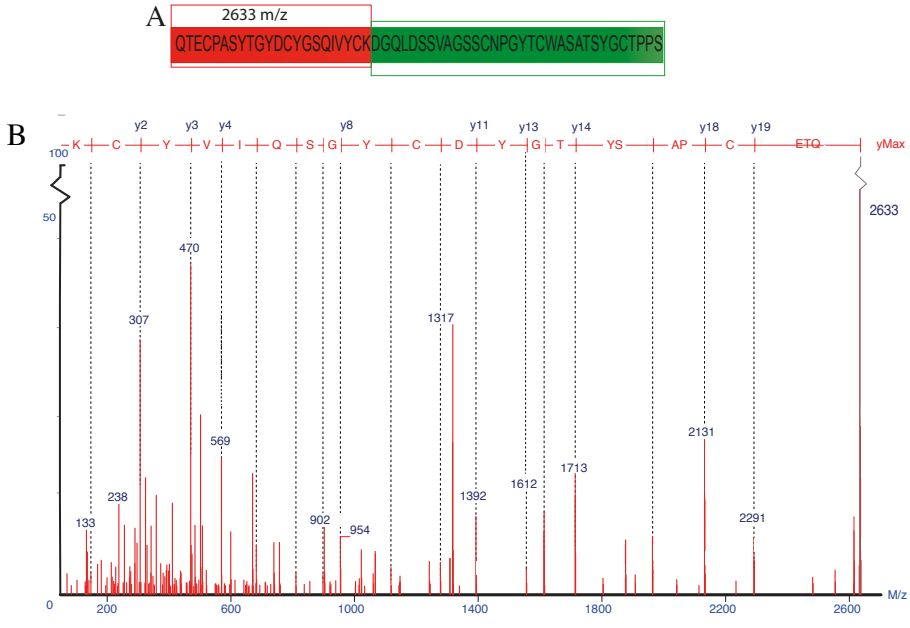

Figure 11. Sequencing and transcriptomic identification of isolated peptide from *Kusaghiporia usambarensis*. (A) MS/MS spectrum of the digested peptides by trypsin resulted in two fragments (only one fragment shown). (B) The peptide fragment where the first seven amino acids were identified and used to search in transcriptome to get complete peptide sequence, which then was automatically mapped to the fragments. All Cysteine residues were modified carbamidomethylation (x time Cys + 57).

By exploiting the kusaghitide sequence as a query in a BLASTp search (Altschul et al. 1997) of the NCBI database, three homologous sequences were retrieved. These sequences were predicted from genomes of *Laetiporus sulphurous* (Nagy et al. 2016), *Wolfiporia cocos* (Floudas et al. 2012) and *Sparassis crispa* (Kiyama et al. 2018) with an identity similarity of 76%, 58% and 53% respectively. The resemblance in peptide sequence correlates with the phylogenetic tree inferred using a three genes multigene dataset (nrLSU, nrSSU and TEF1) (Fig. 12 A-C), insofar that *K. usambarensis* is the closest relative of *L. sulphurous* followed by *W. cocos,* both belonging to the Laetiporaceae (Hussein et al. 2018), while *Sparassis crispa* (in Sparassidaceae) is more remotely related. Thus the predicted protein sequence similarities are congruent with the inferred phylogeny, and all four species are in the 'antrodia clade' (Justo et al. 2017). The fact that similar sequences to the kusaghitide only have been described as coding for a hypothetical protein, makes this study the first evidence for the isolation of this peptide.

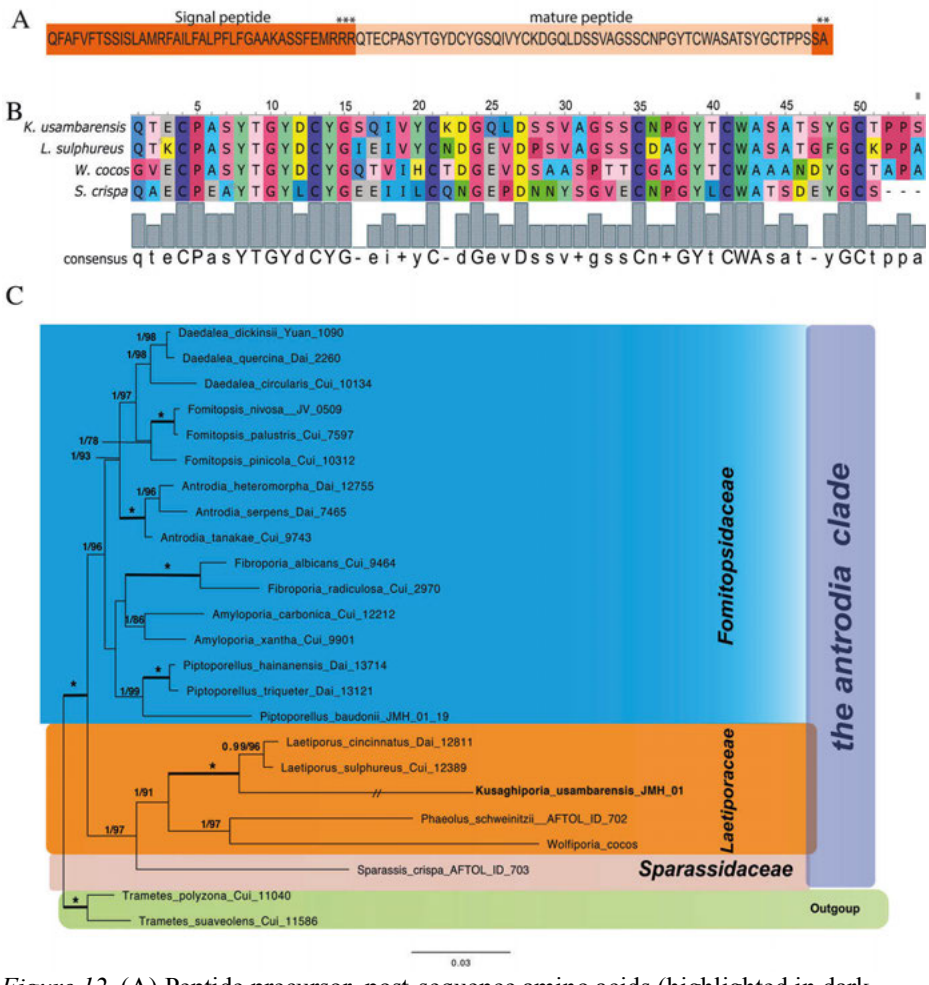

Figure 12. (A) Peptide precursor, post-sequence amino acids (highlighted in dark-brown and marked **) a mature peptide marked (highlighted with pale-brown and *** pre-sequence cleavage site. (B) MAFFT alignment of mature peptide coding sequences retrieved from a BLASTp search and that of kusaghitide using the software Unipro UGENE (Okonechnikov et al. 2012). (C) A maximum likelihood tree illustrating the phylogeny of *K. usambarensis* and its related genera in the 'antrodia clade' based on a combined sequences dataset of nrLSU + nrSSU + TEF1. The tree was rooted with two species from the Polyporaceae. Branches are labelled with maximum likelihood bootstrap (MLbs) higher than 50 % and Bayesian posterior probabilities (*PP*) more than 0.95. Branches in bold indicate support of $PP \geq 0.95$ and MLbs ≥ 70%. An asterisk above a bold branch indicates 100% for both support estimates. Stars indicates mushroom species with homologous peptide sequences. The branch with double-slash is shortened.

The NMR spectroscopy analysis was carried out on a Bruker 600 MHz spectrometer. Isolated peptides were dissolved in 90% H_2O and 10% D_2O. One-dimensional (1D) 1H-NMR experiment was performed prior to homonuclear 1H-1H TOCSY, and NOESY, as well as heteronuclear natural abundance 1H-

15N HSQC. For three-dimensional (3D) analysis, expressed peptide labeled with $^{15}N/^{13}C$ was dissolved in 100% D_2O and 3D heteronuclear experiments including HNCαCβ , HNCα, HNCαCO and (Hβ)CβCα (CO)NNH as well as 2D 1H-15N HSQC were conducted.

The overlaid NOESY and TOCSY spectra (Fig. 13) demonstrate the sequential walk from the Hα of Thr46 connecting to a cross-peak of Hα Ala45 then Ser44, the sequential walk continue up to Gly38. The spectra also show assignments of other residues in the peptide. The assignment of the backbone and side chains resonances were used to calculate the 3D structure of the peptide (Fig. 14). The preliminary peptide structures show three disulfide bonds with Cys4 connected to Cys21, Cys13 to Cys41 and Cys35 connected to Cys50.

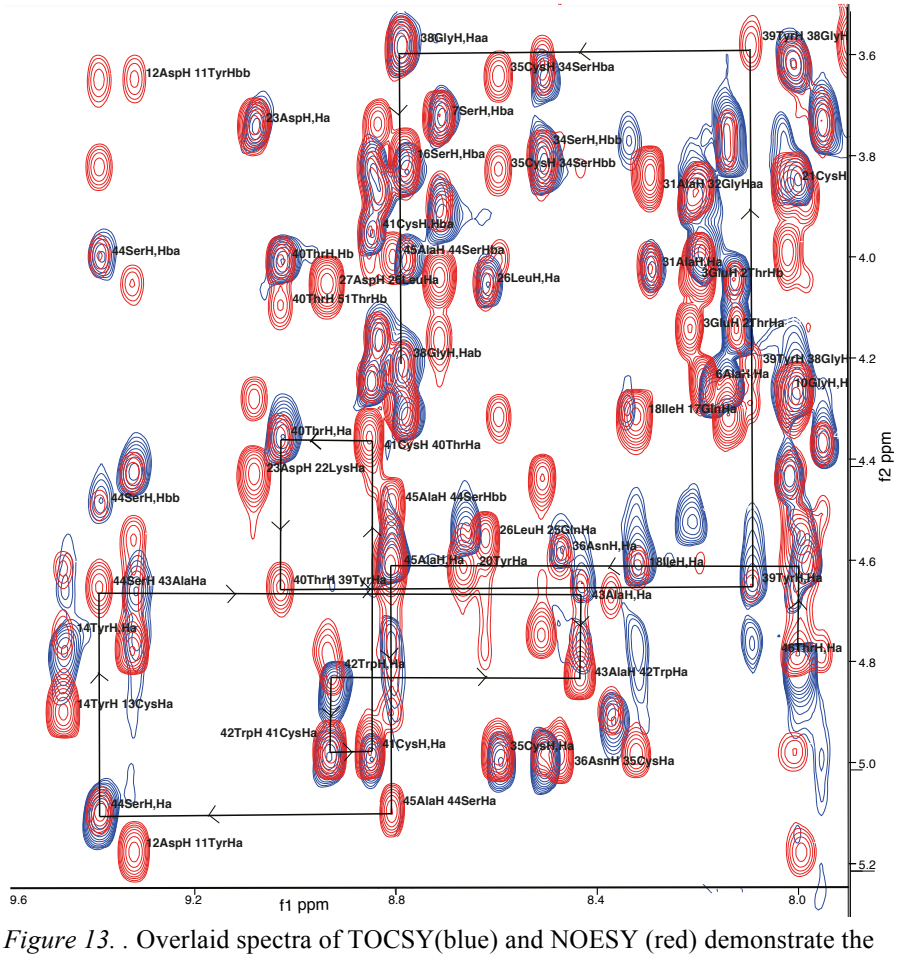

Figure 13. . Overlaid spectra of TOCSY(blue) and NOESY (red) demonstrate the sequential walk from the Hα of Thr46 connecting to a cross-peak of Hα Ala45 up to $H\alpha_a$ and $H\alpha_b$ Gly38.

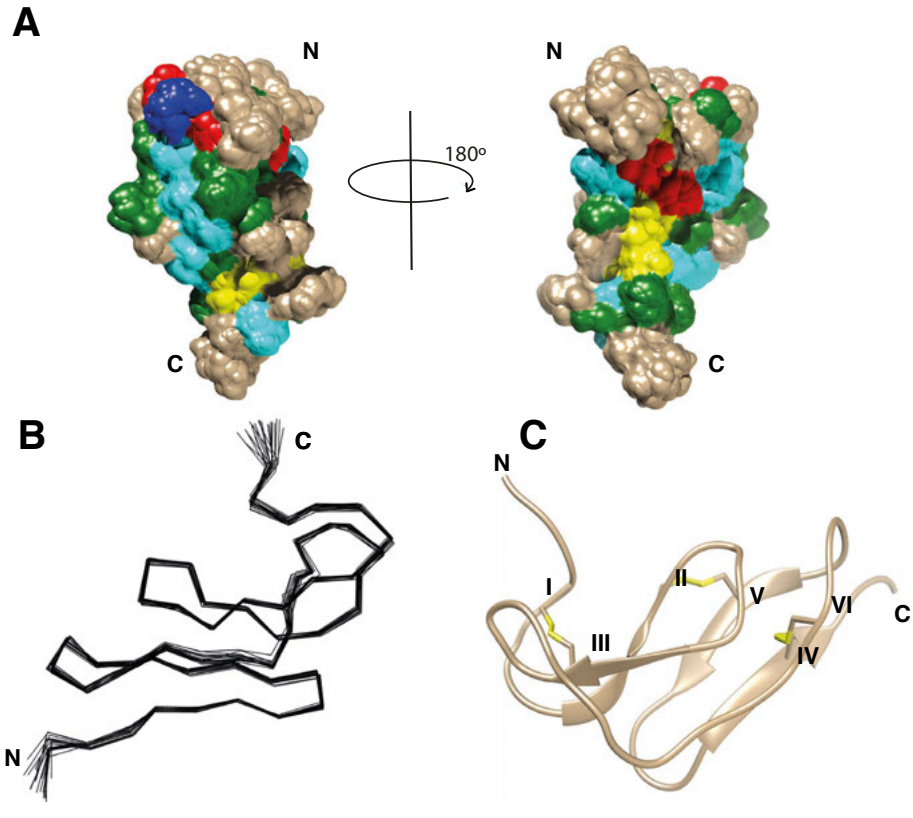

Figure 14. Preliminary 3D structures of kusaghitide. Surface model. (A) Basic residues are shown in blue, acidic red hydrophobic in green, aromatic in cyan and cysteine in yellow (B) Overlay of the 20 models with lowest energy (backbone and heavy atom RMSD 0.47 ± 0.11 Å and 0.80 ± 0.09 Å respectively). (C) Ribbon representation with disulfides (yellow bonds, labeled with roman numbers) C and N-terminal displayed.

Summary of Paper IV: Elucidating the phylogeny of pathogenic fungus *Piptoporellus baudonii*

Tibuhwa, D.D., Hussein, J.M., Ryvarden, L., Sijaona, M.E.R, Tibell, S. (2020). Elucidating the phylogeny of the serious plant pathogen *Piptoporellus baudonii* using a multigene molecular dataset (submitted).

A wide-spread death of trees was observed to be associated with the manifestation of a bright orange-yellow poroid macro-fungus at the bases of trunks and on the ground among the trees (Fig. 15). These fruiting bodies developed from pseudosclerotial tissue composed of a mat of coarse creamy-yellowish mycelium and sand grains that cover the roots and underground parts of the stems of afflicted trees (Westhuizen 1973). Early symptoms of infection are loss of the leaves' deep green color, followed by massive and rapid wilting of the leaves (Rattan and Pawsey 1981). The fungus was described to be soil-borne (Ofosu-Asiedii 1975) and hypothesized to be transmitted through the soil by root contact and by air born-spores (Westhuizen 1973). Infections have been reported from various parts of Africa and include; Congo (Patouillard 1914), Madagascar on *Manihot* sp., and *Cajanus cajan* (as *Phaeolus manihotis* Heim; Heim 1931), Ghana on *Cassia siamea, Khaya senegalensis, Eucalyptus and Citrus* species (Ofosu-Asiedu 1975), Nigeria and the Ivory coast on *Glemina arborea* (Brunck 1962; Rattan and Pawsey 1981), Malawi on tea Plantations (Rattan and Pawsey 1981), South Africa and Swaziland on *Eucalyptus* species (Westhuizen 1973), Kenya on *Acacia tortilis* and Tanzania on *Anacardium occidentale* (Cashew tree), *Manihot* sp. and various indigenous plants (Sijaona 2007).

The taxonomy of the species has remained unstable since it was first described from Congo as *Polyporus baudonii* Pat. (Patouillard 1914). Later studies have referred to the fungus with different names such as *Phaeolus manihotis* R. Heim (Heim 1931), '*Ganoderma colossum*' (Lückhoff 1955), *Phaeolus manihotis* (Browne 1968) and *Laetiporus baudonii* (Pat.) Ryvarden (Ryvarden 1991). In this paper, we ascertained the position of *L. baudonii* by resolving its phylogenetic position using a four gene dataset 5.8S ribosomal RNA loci (rDNA), 28S rDNA (nrLSU) and 18S rDNA (nrSSU), and portions of genes encoding the translation elongation factor 1-α (TEF1) of *L. baudonii* for comparisons with additional sequences from the 'antrodia clade'.

Including additional sequences downloaded from GeneBank, the analyses contained a total of 249 sequences representing 64 species of the 'antrodia clade', with two species from the 'core polyporoid' clade as out-group. The analysis of the concatenated dataset retrieved a phylogeny with two distinct clades representing Fomitopsidaceae and Laetiporaceae, where *Laetiporus baudonii* was found in *Piptoperellus* along with the other three members of

the genus; *P. soloniensis, P. hainanensis*, and *P. triqueter* (Fig. 16). Both morphological and phylogenetic evidence justify the incorporation of *L. baudonii* in *Piptoporellus* as the new combination *Piptoporellus baudonii*.

Figure 15 Piptoporellus baudonii (A) basidioma formed at the base of an eucalyptus tree.

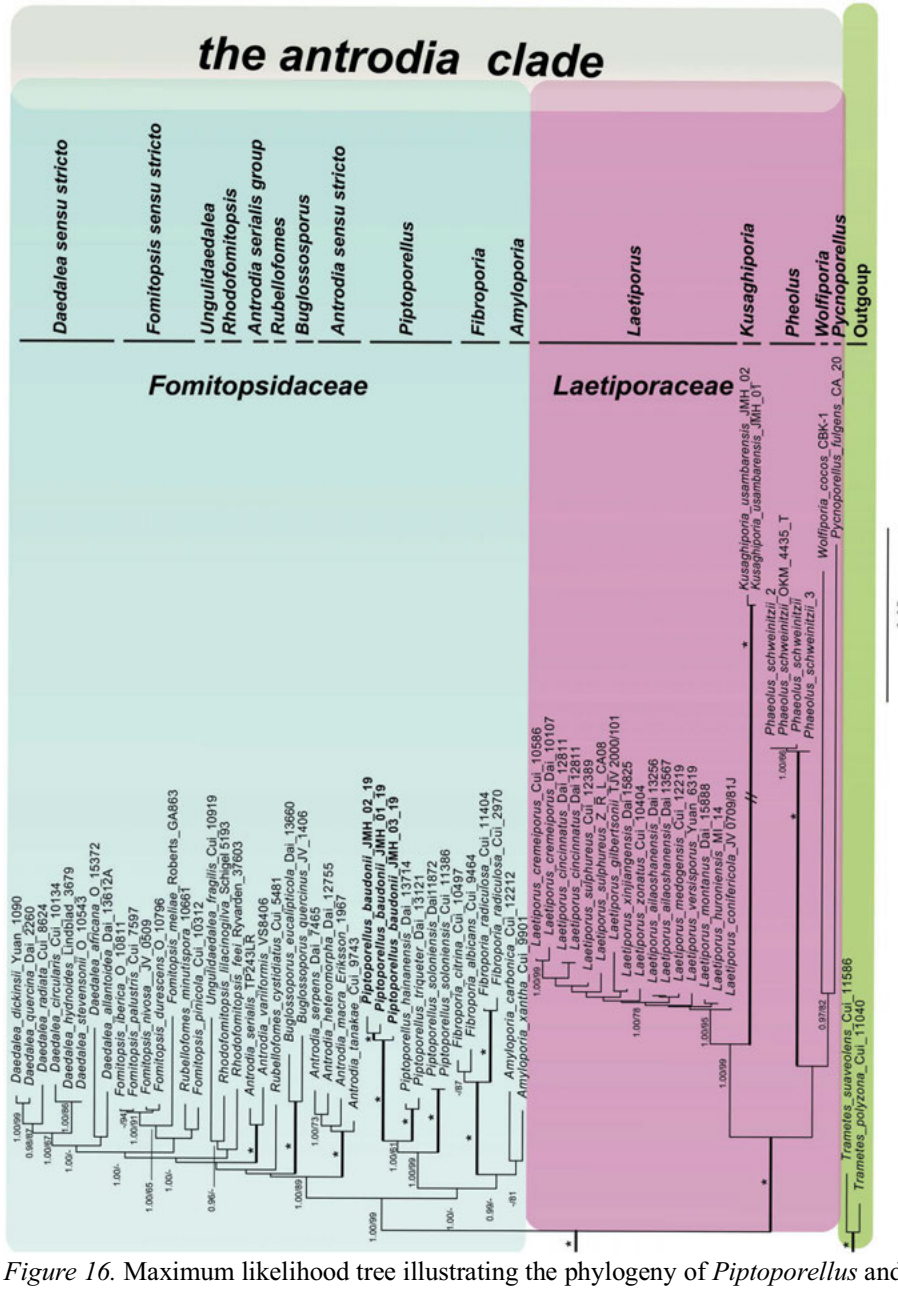

Figure 16. Maximum likelihood tree illustrating the phylogeny of *Piptoporellus* and its related genera in the 'antrodia clade' based on a combined sequences dataset of 5.8S + nrLSU + nrSSU + TEF1. The tree was rooted with two species from the Polyporaceae. Branches are labelled with maximum likelihood bootstrap (MLbs) higher than 50 % and Bayesian posterior probabilities (*PP*) more than 0.95. Branches in bold indicate a support of $PP \geq 0.95$ and MLbs $\geq 70\%$. An asterisk above a bold branch indicates 100% for both support estimates. The branch with double-slash is shortened. Clade names follow Han et al. (2016).

Concluding remarks

The work presented in this thesis contributes to the description of the new genus and new species of the medicinal mushroom *Kusaghiporia usambarensis* and reveals its content of some bioactive compounds. It further elucidated the phylogenetic position of the serious plant pathogen *Piptoporellus baudonii*. The thesis consists of four papers, which aim to investigate polyporoid fungi (*K. usambarensis and Piptoporellus baudonii*) from Tanzania by using morphological and molecular methods combined with biochemical analyses.

A phylogenetic analyses based on a concatenated four genes dataset showed that *K. usambarensis* is closely related to *Laetiporus*, *Phaeolus* and *Wolfiporia* which together form the strongly supported clade of Laetiporaceae. Results from sequence comparisons via BLAST searches in GenBank and the morphological uniqueness of *K. usambarensis* justified its description as new species in a new genus. The information obtained from the indigenous people of the Usambara Mountains on the medicinal usage of this fungus was interesting and inspired further investigations of bioactive compounds. The traditional knowledge of indigenous people should not be underestimated since it is based on a rich practice of traditional medicines and therefore supply a basis for further in-depth studies. *Kusaghiporia* produces large fruiting bodies and is a rare species searched for by members of the local community, hence there is a need for its domestication and also to search for optimal conditions and substrates for its cultivation.

Investigations of the chemical content of *Kusaghiporia* revealed the presence of phenolic compounds, unsaturated fatty acids and their esters. Detection of these compounds may explain the previously reported antioxidant activities and explain the traditional medicinal usage of the species. Degradation of fatty acids resulting in the formation of compounds such as 1-octen-3-one, octanal, 3-octen-2-one contribute to the cheesy, fruity and chicken-like flavour of *Kusaghiporia*. The good flavour, its great size and medicinal properties make *K. usambarensis* desirable for people.

The discovery and characterization of the cysteine-rich peptide, kusaghitide was exciting. Incorporating the transcriptome in the analysis, not only helped to speed up the search for its structure, but also provided additional information on post-translation modification, precursor sequences and cleavage sites. Homologous sequences similar to that of kusaghitide were also found in *Laetiporus*, *Wolfiporia* and *Sparassis* which are phylogenetically related, but these sequences were previously only considered as coding for hypothetical proteins. Kusaghitide was thus the first cysteine-rich peptide to be characterized. The investigations have demonstrated an alternative use of transcriptomic data to improve the experimental data analysis in addition to facilitating genome annotation and quantification of differential gene expression.

The phylogenetic position of the severe pathogen *Piptoporellus baudonii* (formally known as *Laetiporus baudonii*) was elucidated by using multigene analyses. Earlier attempts to describe the fungus relying on morphological data only has created some taxonomic ambiguity. Phylogenetic analyses supported the inclusion of the fungus in *Piptoperellus* along with the other three members of the genus; *P. soloniensis, P. hainanensis*, and *P. triqueter*. Both morphological and phylogenetic evidence justify the inclusion of *L. baudonii* in *Piptoporellus* under the new combination *Piptoporellus baudonii*. Elucidation of the phylogenetic position is a first step towards an understanding of this pathogenic fungus. It presently poses a serious economic challenge since it affects a wide range of hosts plants and is widely distributed in Africa including; Congo, Madagascar, Ghana, Nigeria, Ivory coast, Malawi, South Africa, Swaziland, Kenya and Tanzania. Thus further studies are urgently needed to propose mitigation measures to control it.

Future perspectives

Pharmacognosy is a multi-disciplinary science investigating chemical structural, biochemical and biological activity of substances of natural origins with a drug potential. It encompasses various fields including phylogenetics, biotechnology, chemistry and '-omics' (Larsson et al. 2008). The research work in this thesis spans over all the major areas of pharmacognosy.

Understanding the phylogeny of *Kusaghiporia* was a crucial step towards further research. It enables a proper reference to the scientific name a condition for communication within the scientific community. Also, the knowledge about closely related species provides a basis for comparison with what is already known about related organisms. Future studies should focus on domestication of *K. usambarensis* and to optimize cultivation conditions to enable a safe availability of this favored mushroom.

Screening of chemical composition using GC-MS was done from total dichloromethane extracts. Future studies would include analyses to isolate additional compounds by using various solvents and to test their biological activities. Biological activities related to lowering blood sugar such as amylase and glucosidase inhibition assays could be included.

The approach used in the discovery of kusaghitide may also be used for any other organism. Future work should focus on the biological function of kusaghitide. Based on chemosystematics assumptions, that closely related organisms share a biogenic collection of marginally different chemical derivatives of a common core structure (Larsson et al. 2008), similar assumptions may be applied to *K. usambarensis* and its related organisms. Interestingly, BLAST analyses showed the presence of a kusaghitide homologous peptide from *Laetiporus*, *Wolfiporia* and *Sparassis*, which are phylogenetically related organisms. *Laetiporus*, *Wolfiporia* and *Sparassis* that are well-known to contain bioactive compounds, and a predictive perspective could be applied for future work with focus on *K. usambarensis.* The transcriptome data used in this thesis may also be used to screen the presence of homologous bioactive peptides/proteins produced by other species, which may lead to the discovery of further bioactive molecules.

The revealed phylogenetic position of *Piptoporus baudonii* supplies an important platform for efforts to control/mitigate it. Future work should focus on its pathogenicity and to find mitigation measures to ameliorate its effect on forestry, agriculture and agroforestry.

Svensk sammanfattning

Med “svampar” – inte minst i svampskogen - menar vi oftast de fruktkroppar av svampar som bildas p olika substrat, vanligen p marken eller p stubbar. Men svampar som organismer är s mycket mer än det – de best r av ett vidsträckt nätverk av tunna tr dar som kallas mycel, varav fruktkropparna bara är en (oftast mindre) del, eller för att använda en analogi: äpplena är inte hela trädet M nga svampar är välsmakande, utgör näringsrik mat och en del inneh ller ämnen med medicinska effekter som varit och fortfarande är väl kända i ett flertal kulturer. “Svampar” som mat är dessutom kolesterolfria, inneh ller viktiga mikronäringsämnen, proteiner, vitaminer, essentiella aminosyror och omättade fetter. *Laetiporus sulphureus* (“chicken of the woods”) är en vitt utbredd ätlig art som förekommer, om än ganska sällsynt, i Afrika; en annan är *Kusaghiporia usambarensis* (kusaghizi) som ges en ing ende behandling i denna avhandling.

Svampar inneh ller fenoliska substanser. Fenoler best r av en aromatisk ring med varierande sidogrupper. En del av dem har immunomodererande och/eller kemopreventiva effekter och fungerar som antioxidanter och/eller antibiotika. Svampar konsumeras ofta direkt som mat eller i traditionell medicin ocks efter torkning och ibland malning. S används t. ex. *Wolfiporia cocos* (fu ling) i kinesisk folkmedicin för att behandla urinvägssjukdomar, för att stimulera mjälten och som lugnande medel.

I uppsatsen I beskrivs s som ny för vetenskapen en svamp med mycket stora fruktkroppar fr n Usambarabergen i Tanzania. Mogna fruktkropparna kan n en storlek av upp till 60 cm och väga 10 kg. Att denna svamp hitintills förblivit okänd för vetenskapen är högst anmärkningsvärt. Den kallas *kusaghizi* av Sambaa-folket, vilket p deras spr k har betydelsen “samlare”. Detta namn terspeglar svampens förm ga att växa s väl p buskars torra och levande blad och även annat biologiskt material som den r kar komma i kontakt med - ibland mer oväntade substrat som ormar och insekter. Svampen är högt skattad som mat bland de lokala samhällena och en enda fruktkropp kan delas mellan upp till fyra familjer. Den är känd för att ha en köttliknande struktur och kycklinglik smak. Utövare av traditionell läkemedelskonst blandar torkad mald *kusaghizi* med varmt vatten i en gröt eller som en brygd mot diabetes och högt blodtryck. Den vetenskapliga beskrivningen av svampen, under namnet *Kusaghiporia usambarensis* har baserats p s väl morfologiska som genetiska karaktärer. Svampens namn har bildats fr n Sambaa-namnet p svampen ’kuzaghizi’ och platsen för dess förekomst “Usambara”.

I uppsatserna II och III har det kemiska inneh llet hos *K. usambarensis* undersökts genom en kombination av flera tekniker; transkriptomanalys, gaskromatografi kopplad med masspektrometri (GC-MS), vätskekromatografi/massspektrometri (LC-MS) och kärnmagnetisk resonans (NMR).

Dessa undersökningar p visade förekomsten av fenoler och deras estrar, vilket kan förklara den sedan tidigare kända antioxidantaktiviteten hos svampen och därmed ocks dess användning inom folkmedicinen. Dessutom kan upptäckten av 1-octen-3-one, octanal, och 3-octen-2-one förklara smaken hos *K. usambarensis*, som beskrivs som ost-artad eller kycklinglik. En ny peptid. kushagitidin, upptäcktes ocks . Denna inneh ller tre disulfidbryggor, vilket ger den en ökad resistens mot enzymatisk nedbrytning och höga temperaturer vilket leder till stabilitet över ett brett pH-intervall. Peptidens gen var högt uttryckt p den molekylära niv n och peptiden förekom i höga koncentrationer i vatten-metanol extrakt.

Den fylogenetiska tillhörigheten för en allvarlig växtparasit (tidigare känd under namnet *Laetiporus baudonii*) undersöktes i uppsatsen IV. Denna parasit angriper ett stort antal b de odlade och vilda växter och leder till stora skadeverkningar inom jord- och skogsbruk i olika delar av Afrika, bl.a. i Nigeria, Ghana, Elfenbenskusten, Kongo, Malawi, Swaziland, Sydafrika, Madagaskar, Kenya och Tanzania. I Tanzanias sydliga regioner Lindi och Mtwara observerades ett omfattande vissnande och sedemera död av cashewträd, kassava och eukalyptus. Dessa grödor utgör ryggraden i provinsernas agrara ekonomi och matförsörjning. Fylogenetiska analyser ledde till inkluderandet av denna art i släktet *Piptoporellus*, under den nya kombinationen *P. baudonii*.

Det för denna avhandling utförda forskningsarbetet har resulterat i beskrivningen av en medicinskt intressant svamp, *Kusaghiporia usambarensis*, s som för vetenskapen ny och tillhörande ett nytt släkte. Bioaktiva substanser hos denna art har isolerats och identifierats genom strukturbestämningar. Vidare har arbetet bidragit till att finna den fylogenetiska tillhörigheten av en allvarlig svampparasit och placera denna i rätt släkte under namnet *Piptoporellus baudonii*. Klargörandet av dess fylogeni utgör en grundläggande förutsättning för fortsatta undersökningar av möjliga tgärder för att hejda dess angrepp.

Acknowledgements

Looking back in 2016 when I started my PhD, It was total darkness, not knowing what to expect in research, what will to happen and how it will end. It was amongst the scaring moments of my life. Being thousands miles away from home to pursue scientific aspiration, it wasn't easy, but thanks to my friends and family for their support and encouragement that helped me to get this far. I was fortunate to have friends and colleagues in Uppsala who made Sweden become my second home, they made the long journey relatively shorter and achievable.

During this journey I have learned many things that will be valuable in my career. Appreciations to my mentors and academic supervisors starting with Donatha Tibuhwa who inspired, encouraged and supported me tirelessly. Donatha, you are remarkable. Thank you for the "germplasm project" ☺ during my bachelor project in 2010 that presented me to the world of mushrooms. Your inspiration, care and guidance have changed my life. Over the past ten years, you have been my professional mentor, counsellor and parental figure. I thank you for the field trips to Usambara mountains for mushroom hunting and for the first trip to Sweden in 2015 which introduced me to Sanja. Thank you Donatha for introducing me to the academic world and your endless support "*Umenifungulia dunia*, *ili niwe ninachotaka*. *Asante sana* ☺".

Sanja Tibell, thank you for your enthusiasm ideas and superb support in my PhD life. You were available physically for discussion, through emails or calls to ensure that I receive immediate assistance continuously. You did not only encouraged me to apply for the courses, conference and workshops but you also supported me financially so that I can participate smoothly. It has been an amazing experience to work with you. I have learned a lot from you. Thank you Sanja for all the time you have devoted to assist me grow as a scientist and as a person ☺. Your current and future students are lucky to have you, I hope our footpaths will continue to interact much more in future.

Christa Wedén, thank you for your advices and tremendous academic support during this entire period of my PhD studies. You have led me to the world of truffles, a breath-taking field which I have fallen in love with. It is a potential I consider for the future. Your constructive opinions has helped me to grow and improve every day. Thank you for reading repetitively drafts of my manuscripts until it looks better, and for always making time for meetings and discussion. Your supportive nature as a supervisor has been critical to my improvements. Thank you Christina ☺.

Ulf Göransson, you are the coolest supervisor ever ☺. You trusted me, inspired and guided me to the right direction. You mentored me in an exciting world of pharmacognosy and peptide chemistry, a field full of fun and an exciting future. You encouraged me when things were tough for me due to my lack of experience, we sat and work together through the challenges and you

proved to me that nothing is impossible when one has a positive attitude. I feel privileged to have had your support. Thank you Ulf ☺. I hope this is the beginning of prosperous future collaborations.
Thanks to my collaborators and co-authors for their contributions, Hesham El-Seed, Chi Celestine, Johan Rosengren, Eric Jacobsson, Mark Sijaona, Frank Mbago and Leif Ryvarden. Thank you for making this possible.
To my colleagues at Systematic Biology program, I am grateful for your support during my journey. Thank you all particularly Hanna, Leif, Martin, Jennifer, Sandie, Nahid, Ioana, Henrik, Magnus, Petra, Mats, Mickel, David and Agneta for all the support, assistance and useful information they have given to me always. Thank you Stella, Diem, Brendan, Iker, Aaron, Mahwash, Anneli, Ivain, Baset, CJ, Faheema, Sandra Lorena, Anders, Julia, Sanea, Raquel, Fabian, Markus, Caesar and Inga, your support is well appreciated.
My colleagues at pharmacognosy group, Thank you for the incredible support to me. I appreciate your patience with me whenever I come to you. I was welcomed and helped me with warm smiles every time I knock to your doors with questions about instruments and methods that I was unfamiliar with. Thank you Luke, Anders, Adam, Paco, Camilla, Taj, Sunithi, Lakmini, Astrid, Karin, Quentin, Scott, Blazej, Vasiliki, Mahmoud, Jayani, Doua, Khaldoun, Islam, Mingshu, Julio and Omar.
I owe big thanks to my colleagues and friends (the team Tz), you guys are awesome. Thank you for the quality time we have spent together. Happy, Lwidiko (Man-Edo), Ally (*mtaalamu wa viungo* ☺), Watson, Japhet, Samya, Grace, Merezia, Pitos, Alice, Maglan, Mesiah, Mohammed, Donath, Ruben, Herieth, Justine, Zuhura, Koku, Nuria, Andreas and Stella. Our network need to be enriched for the future.
To all members of the department of Molecular biology and biotechnology (MBB family) at the University of Dar es Salaam. Thank you for taking extra workloads of teaching and other duties for us who pursued further studies. Thank you Dr. Kassuwi, Shamte, Shimba, Prof. Lymo, Prof. Kivaisi, Dr. Prosper, Marietha, Prof. Magingo, Dr. Masalu, Fredinad, Dr. Makene, Dr. Mahadhi, Prof. Mshandete, Dr. Modesta, Dr. Mpinda, Dr. Mariam, Aneth, Charles, Winnie, Kelvin, Dr. Temu, Shauri, and Hawa.
Kwa wazazi wangu, Sheikh Shawal, Sheikh Suleiman, Mama Daudo ☺, Mamaa Ukwmani ☺, na Shangazi, asanteni sana. Upendo wenu, maombi na dua zenu. Nyinyi ni silaha kubwa kwangu na nguzo ya mafanikio yangu. Ushauri, malezi na nasha zenu ndizo zimenifikisha hapa. Naomba Mwenyezi mungu awalinde na kuwahifadhi ili tuendelee kufaidi matunda ya uwepo wenu. Aamin.
Kwa ndugu zangu, Kaka Kalungo*,* Bro Jimmy (*mwarabu wa* Dubai ☺)*,* Malik, *Da Ki a, Da* Fatma*, Da* habiba*, Da* Rehema*,* Nasra*,* Dalila*,* Chance*,* Hassan*,* Eric*, Da* Siwa *na bwan-shem i* Khamis ☺, *mmenifari i na kunipa moyo kuwa inawezekana, asanteni*. To my nieces and nephews *(*team *butumburas) wote*

☺, *asanteni. Kwa hakika nyinyi ni hazina kubwa kwangu, jiandaeni kupokea kijiti kutoka kwa Ankal* ☺.
I am grateful to thanks to SIDA project no. UDSM – SIDA programme 2015 – 2020: Project No. 222 for financial support which without it the project wouldn't be feasible.
My lovely wife Mwam, thank you for not stop supporting me, for being my friend, partner, companion and much more. I could not have done it without you. Thank you hany ☺. *Wewe ni jembe*, *mpambanaji,* Kish *wewe ni Mama lao* ☺.
Lastly, to my adorable kids Sakina and Saira *furaha ya moyo wangu* , thank you for being such a wonderful gift to me. In those cold and long-dark days of Sweden, your warm smiles and charming faces illuminated my days and gave me strength to not giving up.
Above all *Alhadulillah* by his grace and blessings. I praise Allah for the grace of life, health, wisdom and knowledge until I have accomplished all this. It was not for my cunning, but for his love and grace. May Allah continue to protect, elevate me and make today better than yesterday and tomorrow a better than today. *Aamin.*
A voyage of thousands miles begin with the first step, this is not the end rather the beginning of an exciting scientific expedition.

References

1. Adenipekun, C.O. and Lawal, R. (2012). Uses of mushrooms in bioremediation: A review. *Biotechnology and Molecular Biology Reviews*, *7*(3), 62–68.
2. Adeoye-Isijola, M.O., Olajuyigbe, O.O., Jonathan, S.G. and Coopoosamy, R.M. (2018). Bioactive compounds in ethanol extract of *Lentinus squarrosulus* Mont. A Nigerian medicinal macrofungus. *African Journal of Traditional, Complementary and Alternative Medicines*, *15*(2), 42–50.
3. Altschul, S.F., Madden, T.L., Schäffer, S., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*, *25*, 3389–3402.
4. Arnott, D. (2001). Basics of triple-stage quadrupole/ion-trap mass spectrometry: precursor, product and neutral-loss scanning, electrospray ionisation and nanospray ionisation. In Proteome Research: Mass Spectrometry (pp. 11–31). Springer, Berlin, Heidelberg.
5. Arora, D. and Hershey, H. (1986). *Mushrooms demystified* (Vol. 23). Berkeley: Ten Speed Press.
6. Arn, H. and Acree, T.E. (1998). Flavornet: a database of aroma compounds based on odor potency in natural products. *Developments in Food Science*, *40*, 27–28.
7. Banik, M.T., Lindner, D.L., Ota, Y. and Hattori, T. (2010). Relationships among North American and Japanese *Laetiporus* isolates inferred from molecular phylogenetics and single-spore incompatibility reactions. *Mycologia*, *102*(4), 911–917.
8. Barros, L., Baptista, P., Correia, D.M., Casal, S., Oliveira, B. and Ferreira, I.C. (2007). Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. *Food Chemistry*, *105*(1), 140–145.
9. Bennet, J.W., Wunch, K.G. and Faison, B.D. (2002). Use of fungi in biodegradation. *Environmental Microbiology. 2nd edition, ASM Press Washington DC*.
10. Bentley, D.R., Balasubramanian, S., Swerdlow, H.P., Smith, G.P., Milton, J., Brown, C.G., ... and Boutell, J.M. (2008). Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*, *456*(7218), 53.
11. Binder, M., Justo, A., Riley, R., Salamov, A., Lopez-Giraldez, F., Sjökvist, E., ... and Larsson, K.H. (2013). Phylogenetic and phylogenomic overview of the Polyporales. *Mycologia*, *105*(6), 1350–1373.
12. Blau, K. and Halket, J.M. (1993). *Handbook of derivatives for chromatography*. Wiley.
13. Bougher, N.L. and Syme, K. (1998). *Fungi of Southern Australia*. University of Western Australia Press.
14. Browne, F.G. (1968). Pests and diseases of forest plantation trees. An annotated list of the principal species occur ring in the British Commonwealth, p. 923. Clarendon Press, Oxford.
15. Brunck, F. (1962). Aperçu sur les principales attaques parasitaires observées dans les plantations foresti res d'Afrique tropicale.
16. Bulaj, G. (2005). Formation of disulfide bonds in proteins and peptides. *Biotechnology advances*, *23*(1), 87–92.
17. Burgess, N., Butynski, T.M., Cordeiro, N.J., Doggart, N.H., Fjelds , J., Howell, K.M., ... and Menegon, M. (2007). The biological importance of the Eastern Arc Mountains of Tanzania and Kenya. *Biological Conservation*, *134*(2), 209–231.

18. Butala, M., Šega, D., Tomc, B., Podlesek, Z., Kem, W.R., Küpper, F.C., & Turk, T. (2015). Recombinant expression and predicted structure of parborlysin, a cytolytic protein from the Antarctic heteronemertine *Parborlasia corrugatus*. *Toxicon*, *108*, 32–37.
19. Byrne, P.F. and Brennan, P.J. (1975). The lipids of *Agaricus bisporus*. *Microbiology*, *89*(2), 245–255.
20. Chang, R. (1996). Functional properties of edible mushrooms. *Nutrition Reviews*, *54*(11), 91–93.
21. Chen, W., Hwang, Y.Y., Gleaton, J.W., Titus, J.K., & Hamlin, N.J. (2018). Optimization of a peptide extraction and LC–MS protocol for quantitative analysis of antimicrobial peptides. *Future science OA*, *5*(1), FSO348.
22. Chibata, I., Okumura, K., Takeyama, S. and Kotera, K. (1969). Lentinacin: a new hypocholesterolemic substance in *Lentinus edodes*. *Experientia*, *25*(12), 1237–1238.
23. Combet, E., Henderson, J., Eastwood, D.C. and Burton, K.S. (2006). Eight-carbon volatiles in mushrooms and fungi: properties, analysis, and biosynthesis. *Mycoscience*, *47*(6), 317–326.
24. Davoli, P., Mucci, A., Schenetti, L. and Weber, R.W. (2005). Laetiporic acids, a family of non-carotenoid polyene pigments from fruit-bodies and liquid cultures of *Laetiporus sulphureus* (Polyporales, Fungi). *Phytochemistry*, *66*(7), 817–823.
25. Eguchi, M., Itoh, M., Nishino, K., Shibata, H., Tanaka, T., Kamei-Hayashi, K. and Hara, S. (1994). Amino acid sequence of an inhibitor from the silkworm (*Bombyx mori*) hemolymph against fungal protease. *The Journal of Biochemistry*, *115*(5), 881–884.
26. El Hadi, M.A.M., Zhang, F.J., Wu, F.F., Zhou, C.H. and Tao, J. (2013). Advances in fruit aroma volatile research. *Molecules*, *18*(7), 8200–8229.
27. Feng, C., Chen, M., Xu, C.J., Bai, L., Yin, X.R., Li, X., ... and Chen, K. S. (2012). Transcriptomic analysis of Chinese bayberry (*Myrica rubra*) fruit development and ripening using RNA-Seq. *BMC genomics*, *13*(1), 19.
28. Fenn, J.B., Mann, M., Meng, C.K., Wong, S.F. and Whitehouse, C.M. (1989). Electrospray ionization for mass spectrometry of large biomolecules. *Science*, *246*(4926), 64–71.
29. Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R.A., Henrissat, B., ... and Aerts, A. (2012). The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science*, *336*(6089), 1715–1719.
30. Gaberc-Porekar, V. and Menart, V. (2001). Perspectives of immobilized-metal affinity chromatography. *Journal of biochemical and biophysical methods*, *49*(1–3), 335–360.
31. Garber, M., Grabherr, M.G., Guttman, M. and Trapnell, C. (2011). Computational methods for transcriptome annotation and quantification using RNA-seq. *Nature methods*, *8(*6), 469.
32. Geron, M. (2020). Production and Purification of Recombinant Toxins. In *Snake and Spider Toxins* (pp. 73–84). Humana, New York, NY.
33. Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., ... and Chen, Z. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature biotechnology*, *29*(7), 644.
34. Gunstone, F.D. and Harwood, J.L. (2007). Occurrence and characterisation of oils and fats. *In The lipid handbook with* CD-ROM (pp. 51–156). CRC press.
35. Hale, J.E., Butler, J.P., Gelfanova, V., You, J.S. and Knierman, M.D. (2004). A simplified procedure for the reduction and alkylation of cysteine residues in proteins prior to proteolytic digestion and mass spectral analysis. *Analytical biochemistry*, *333*(1), 174–181.

36. Hall, J., Burgess, N.D., Lovett, J., Mbilinyi, B. and Gereau, R.E. (2009). Conservation implications of deforestation across an elevational gradient in the Eastern Arc Mountains, Tanzania. *Biological Conservation*, *142*(11), 2510–2521.
37. Hamilton, R.J. and Sewell, P.A. (1982). Introduction to high performance liquid chromatography. In *Introduction to high performance liquid chromatography* (pp. 1–12). Springer, Dordrecht.
38. Heim R. (1931). Le *Phaeolus manihotis* sp. nov., parasite du manioc a Madagascar, et considérations sur le genre *Phaeolus* Pat. *Annales de Cryptogamie Exotique*, *4*:175–189.
39. Henschen, A. (1986). Analysis of cyst (e) ine residues, disulfide bridges, and sulfhydryl groups in proteins. In *Advanced methods in protein microsequence analysis* (pp. 244–255). Springer, Berlin, Heidelberg.
40. Hibbett, D.S. (2007). After the gold rush, or before the flood? Evolutionary morphology of mushroom-forming fungi (Agaricomycetes) in the early 21st century. *Mycological Research*, *111*(9), 1001–1018.
41. Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E., Huhndorf, S., James, T., Kirk, P.M., Lücking, R. and Lumbsch, H.T. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research*, *111*(5), 509–547.
42. Huang, C.B., Alimova, Y., Myers, T.M. and Ebersole, J.L. (2011). Short-and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Archives of oral biology*, *56*(7), 650–654.
43. Hussein, J.M., Tibuhwa, D.D. and Tibell, S. (2018). Phylogenetic position and taxonomy of *Kusaghiporia usambarensis* gen. et sp. nov. (Polyporales). *Mycology*, 1–9.
44. https://abrf.org/delta-mass.
45. http://www.emapsworld.com/tanzania-regions-map-black-and-white.html
46. http://open_jicareport.jica.go.jp/pdf/11681558_02.PDF.
47. Juma, I., Mshandete, A.M., Tibuhwa, D.D. and Kivaisi, A. (2016). Assessment of antioxidant potentials of the wild and domesticated saprophytic edible mushrooms from Tanzania. *Curr Res Environ App Mycol*, *6*, 1–10.
48. Justo, A., Miettinen, O., Floudas, D., Ortiz-Santana, B., Sjökvist, E., Lindner, D., Nakasone, K., Niemelä, T., Larsson, K.H., Ryvarden, L. (2017). A revised family-level classification of the Polyporales (Basidiomycota). *Fungal Biology*. *9*:798–824.
49. Kabir, Y., Kimura, S. and Tamura, T. (1988). Dietary effect of *anoderma lucidum* mushroom on blood pressure and lipid levels in spontaneously hypertensive rats (SHR). *Journal of Nutritional Science and Vitaminology*, *34*(4), 433–438.
50. Karlsson, O.A., Ramirez, J., berg, D., Malmqvist, T., Engström, Å., Friberg, M., ... and Jemth, P. (2015). Design of a PDZbody, a bivalent binder of the E6 protein from human papillomavirus. *Scientific reports*, *5*, 9382.
51. Kanelis, V., Forman-Kay, J.D. and Kay, L.E. (2001). Multidimensional NMR methods for protein structure determination. *IUBMB life*, *52*(6), 291–302.
52. Kirk, P.P., Cannon, D., Minter and J. Stalpers (2008). Ainsworth and Bisby's Dictionary of the Fungi, 10th edition. CABI, Wallingford, UK.
53. Kitson, F.G., Larsen, B.S. and McEwen, C.N. (1996). Gas chromatography and mass spectrometry: a practical guide. Academic Press.
54. Kiyama, R., Furutani, Y., Kawaguchi, K. and Nakanishi, T. (2018). Genome sequence of the cauliflower mushroom *Sparassis crispa* (Hanabiratake) and its association with beneficial usage. *Scientific reports*, *8*(1), 1–11.

55. Krishnamoorthy, K. and Subramaniam, P. (2014). Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. *International scholarly research notices*, 2014.
56. Kwan, A.H., Mobli, M., Gooley, P.R., King, G.F. and Mackay, J.P. (2011). Macromolecular NMR spectroscopy for the non-spectroscopist. *The FEBS ournal*, *278*(5), 687–703.
57. Lai, K.H., Lu, M.C., Du, Y.C., El-Shazly, M., Wu, T.Y., Hsu, Y.M., ... and Wu, Y.C. (2016). Cytotoxic lanostanoids from *Poria cocos*. *Journal of natural products*, *79*(11), 2805–2813.
58. Larsson, S., Backlund, A. and Bohlin, L. (2008). Reappraising a decade old explanatory model for pharmacognosy. *Phytochemistry Letters*, *1*(3), 131–134.
59. Larsson, K.H., Larsson, E. and K ljalg, U. (2004). High phylogenetic diversity among corticioid homobasidiomycetes. *Mycological Research*, *108*(9), 983–1002.
60. León, F., Quintana, J., Rivera, A., Estévez, F. and Bermejo, J. (2004). Lanostanoid Triterpenes from *Laetiporus sulphureus* and Apoptosis Induction on HL-60 Human Myeloid Leukemia Cells. *Journal of Natural Products*, *67*(12), 2008–2011.
61. Lindner, D.L. and Banik, M.T. (2008). Molecular phylogeny of *Laetiporus* and other brown rot polypore genera in North America. *Mycologia*, *100*(3), 417–430.
62. Longvah, T. and Deosthale, Y.G. (1998). Compositional and nutritional studies on edible wild mushroom from northeast India. *Food Chemistry*, *63*(3), 331–334.
63. Lonsdale, D., Pautasso, M. and Holdenrieder, O. (2008). Wood-decaying fungi in the forest: conservation needs and management options. *European Journal of Forest Research*, *127*(1), 1–22.
64. Lovett, J.C. (1990). Classification and status of the moist forests of Tanzania. *Mitteilungen aus dem Institut f r Allgemeine Botanik Hamburg*, 287–300.
65. Lückhoff, H.A. (1955). Two hitherto unrecorded fungal diseases attacking Pines and Eucalyptus in South Africa. *Journal of South African Forestry Association*, *26*:47–61.
66. Marx, F. (2004). Small, basic antifungal proteins secreted from filamentous ascomycetes: a comparative study regarding expression, structure, function and potential application. Applied microbiology and biotechnology, *65*(2), 133–142.
67. Mortazavi, A., Williams, B.A., McCue, K., Schaeffer, L. and Wold, B. (2008). Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nature methods*, *5*(7), 621.
68. Mosciano, G., Fasano, M., Cassidy, J., Connelly, K., Mazeiko, P., Montenegro, A., ... and Michalski, J. (1993). Organoleptic characteristics of flavor materials. *Perfumer flavorist*, *18*(1), 43–45.
69. Muruke, M.H. (2014). Evaluation of antioxidant and iron chelating activities of a wild edible oyster mushroom *Pleurotus cystidiosus* from Tanzania. *Evaluation*, *29*.
70. Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A. and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*(6772), 853.
71. Nagy, L.G., Riley, R., Tritt, A., Adam, C., Daum, C., Floudas, D., ... and Matsuura, K. (2016). Comparative genomics of early-diverging mushroom-forming fungi provides insights into the origins of lignocellulose decay capabilities. *Molecular biology and evolution*, *33*(4), 959–970.
72. Nichols, D.S. and Sanderson, K. (2003). The nomenclature, structure, and properties of food lipids. *Chemical and functional properties of food lipids*, 29–59.
73. Ofosu-Asiedu, A. (1975). A new disease of eucalypts in Ghana. *Transactions of the British Mycological Society*, *65*:285–289.
74. Okonechnikov, K., Golosova, O., Fursov, M. and Ugene Team. (2012). Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics*, *28*(8), 1166–1167.

75. Olombrada, M., Lázaro-Gorines, R., López-Rodríguez, J.C., Martínez-del-Pozo, Á., O aderra, M., Maestro-López, M., ... and García-Ortega, L. (2017). Fungal ribotoxins: a review of potential biotechnological applications. *Toxins*, *9*(2), 71.
76. Palacios, I., Lozano, M., Moro, C., D'arrigo, M., Rostagno, M.A., Martínez, J.A., ... and Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chemistry*, *128*(3), 674–678.
77. Pandit, N.P. and Maheshwari, S.K. (2012). Optimization of cellulase enzyme production from sugarcane pressmud using oyster mushroom *Pleurotus sa or-ca u* by solid state fermentation. *Journal of Bioremed and Biodegrad*, *3*(3), 1–5.
78. Patouillard, N.T. (1914). Quelques champignons du Congo. *Bulletin de la Soci t mycologique de France*, *30*:336–346.
79. Platts, P.J., Burgess, N.D., Gereau, R.E., Lovett, J.C., Marshall, A.R., McClean, C.J., ... and Marchant, R. (2011). Delimiting tropical mountain ecoregions for conservation. *Environmental Conservation*, *38*(3), 312–324.
80. Papayannopoulos, I.A. (1995). The interpretation of collision-induced dissociation tandem mass spectra of peptides. *Mass spectrometry reviews*, *14*(1), 49–73.
81. Petrova, A., Alipieva, K., Kostadinova, E., Antonova, D., Lacheva, M., Gjosheva, M., ... and Bankova, V. (2007). GC-MS studies of the chemical composition of two inedible mushrooms of the genus *Agaricus*. *Chemistry Central Journal*, *1*(1), 33.
82. Pinho, P.G.D., Ribeiro, B., Gonçalves, R.F., Baptista, P., Valentão, P., Seabra, R.M. and Andrade, P. B. (2008). Correlation between the pattern volatiles and the overall aroma of wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, *56*(5), 1704–1712.
83. Pope, B. and Kent, H.M. (1996). High efficiency 5 min transformation of Escherichia coli. *Nucleic acids research*, *24*(3), 536–537.
84. Powell, B. (2012). Biodiversity and human nutrition in a landscape mosaic of farms and forests in the East Usambara Mountains, Tanzania (Doctoral dissertation, McGill University Libraries).
85. Rattan, P.S. and Pawsey, R.G. (1981). Death of tea in Malawi caused by *Pseudophaeolus baudonii*. *International Journal of Pest Management*, *27*(2), 225–229.
86. Razaq, A., Khalid, A.N. and Ilyas, S. (2013). Molecular Identification of *Lepiota acutesquamosa* and *L. cristata* (Basidiomycota, Agaricales) based on ITS-rDNA Barcoding from Himalayan Moist Temperate Forests of Pakistan. *International Journal of Agriculture Biology*, *15*(2).
87. Risticevic, S., Niri, V.H., Vuckovic, D. and Pawliszyn, J. (2009). Recent developments in solid-phase microextraction. *Analytical and bioanalytical chemistry*, *393*(3), 781–795.
88. Sijaona, M.E.R., Shija B. (2005). Verification of Bayleton 25WP for the control of powdery mildew (*Oidium anacardii* F. Noack) on cashew in Tanzania. Registration report submitted to Registrar of pesticides TPRI, Arusha-Tanzania.
89. Suseem, S.R. and Saral, A.M. (2013). Analysis on essential fatty acid esters of mushroom *Pleurotus eous* and its antibacterial activity. *Asian Journal Pharmacy and Clinical Research*, *6*(1), 188–91.
90. Rice, P., Longden, I. and Bleasby, A. (2000). EMBOSS: the European molecular biology open software suite. *Trends enet*, *16*(6):276–7.
91. Ryvarden, L. (1991). Genera of polypores, nomenclature and taxonomy. Synopsis *Fungorum* (Oslo) *5*:1–373.
92. Salem, M.A., Jüppner, J., Bajdzienko, K. and Giavalisco, P. (2016). Protocol: a fast, comprehensive and reproducible one-step extraction method for the rapid preparation of polar and semi-polar metabolites, lipids, proteins, starch and cell wall polymers from a single sample. *Plant methods*, *12*(1), 45.

93. Schroeder, C.I. and Rosengren, K.J. (2020). Three-Dimensional Structure Determination of Peptides Using Solution Nuclear Magnetic Resonance Spectroscopy. In *Snake and Spider Toxins* (pp. 129–162). Humana, New York, NY.
94. Steen, H. and Mann, M. (2004). The ABC's (and XYZ's) of peptide sequencing. *Nature reviews Molecular cell biology*, *5*(9), 699–711.
95. Sijaona, M.E.R. (2007). Macro-fungi in Association with Cashew Wilt disease in Tanzania. A poster presented at the 18th Congress of the Association for the Taxonomic study of the Flora of Tropical Africa [AETFAT], held between 26 February to 02 March, 2007; in Yaounde, Cameroon.
96. Sletten, K. and Gran, L. (1973). Some molecular properties of kalatapeptide B-1. A uterotonic polypeptide isolated from *Oldenlandia affinis* DC. *Medd Nor Farm Selsk*, *7*(8), 69–82.
97. Tangvarasittichai, S., Tangvarasittichai, O. and Jermnim, N. (2009). Comparison of fast protein liquid chromatography (FPLC) with HPLC, electrophoresis & microcolumn chromatography techniques for the diagnosis of β-thalassaemia. *Indian Journal of Medical Research*, *129*(2), 2–2.
98. Tao, N., Jia, L. and Zhou, H. (2014). Anti-fungal activity of *Citrus reticulata* Blanco essential oil against *Penicillium italicum* and *Penicillium digitatum*. *Food chemistry*, *153*, 265–271.
99. Taylor, D.L., Hollingsworth, T.N., McFarland, J.W., Lennon, N.J., Nusbaum, C. and Ruess, R.W. (2014). A comprehensive census of fungi in soil reveals both hyperdiversity and ne-scale niche partitioning. *Ecological Monographs, 84*, 3–20.
100. Tóth, L., Kele, Z., Borics, A., Nagy, L.G., Váradi, G., Virágh, M., ... and Galgóczy, L. (2016). NFAP2, a novel cysteine-rich anti-yeast protein from *Neosartorya fischeri* NRRL 181: isolation and characterization. *AMB Express*, *6*(1), 75.
101. Turkoglu, A., Duru, M.E., Mercan, N., Kivrak, I. and Gezer, K. (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chemistry*, *101*(1), 267–273.
102. Wasser, S.P., Sokolov, D., Reshetnikov, S.V. and Timor-Tismenetsky, M. (2000). Dietary supplements from medicinal mushrooms: diversity of types and variety of regulations. *International Journal of Medicinal Mushrooms*, *2*(1).
103. Van der Westhuizen, G.C.A. (1973). *Polyporus baudoni* Pat. on *Eucalyptus* spp. in South Africa. *Bothalia*, *11*(1/2), 143–151.
104. Weiss, H.B. (1920). The insect enemies of polyporoid fungi. *The American Naturalist*, *54*(634), 443–447.
105. Wellner, D., Panneerselvam, C. and Horecker, B.L. (1990). Sequencing of peptides and proteins with blocked N-terminal amino acids: N-acetylserine or N-acetylthreonine. *Proceedings of the National Academy of Sciences*, *87*(5), 1947–1949.
106. Wu, H.X., Jia, H.M., Ma, X.W., Wang, S.B., Yao, Q.S., Xu, W.T., ... and Zhan, R.L. (2014). Transcriptome and proteomic analysis of mango (*Mangifera indica* Linn) fruits. *Journal of proteomics*, *105*, 19–30.
107. Wu, T.D. and Watanabe, C.K. (2005). GMAP: a genomic mapping and alignment program for mRNA and EST sequences. *Bioinformatics*, *21*(9), 1859–1875.
108. Wüthrich, K. (1986). NMR with proteins and nucleic acids. *Europhysics News*, *17*(1), 11–13.
109. Yu, F.R., Lian, X.Z., Guo, H.Y., McGuire, P.M., Li, R.D., Wang, R. and Yu, F.H. (2005). Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. *Journal of Pharmacy and Pharmaceutical Sciences*, *8*(3), 528–35.
110. Zhao, C.L., Cui, B.K., Song, J. and Dai, Y.C. (2015). Fragiliporiaceae, a new family of Polyporales (Basidiomycota). *Fungal Diversity*, *70*(1), 115–126.

111. Zhu, J.S., Halpern, G.M., and Jones, K. (1998). The scientific rediscovery of an ancient Chinese herbal medicine: *Cordyceps sinensis* Part I. *The Journal of Alternative and Complementary Medicine*, *4*(3), 289–303.
112. Zmitrovich, I.V., Malysheva, V.F. and Spirin, W.A. (2006). A new morphological arrangement of the Polyporales. I. Phanerochaetineae. *Mycena*: an independent mycological journal, (6), 4–56.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 1908

Editor: The Dean of the Faculty of Science and Technology

A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-405548

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020