



UPPSALA
UNIVERSITET

*Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Science and Technology 1979*

Lichens in Mountain Rainforests of Tanzania

Studies of Usnea and Calicioids

STELLA TEMU



ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020

ISSN 1651-6214
ISBN 978-91-513-1043-5
urn:nbn:se:uu:diva-422950

Dissertation presented at Uppsala University to be publicly examined in Ekmansalen, Evolutionsbiologiskt centrum (EBC), Norbyvägen 14-18, UPPSALA, Wednesday, 28 April 2021 at 10:00 for the degree of Doctor of Philosophy. The examination will be conducted in English. Faculty examiner: Professor Einar Timdal (University of Oslo.).

Abstract

Gilbert Temu, S. 2020. Lichens in Mountain Rainforests of Tanzania. Studies of *Usnea* and Calicioids. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 1979. 50 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-1043-5.

Lichens occur in various habitats. They often have narrow niches and are sensitive to environmental changes leading to their use as bioindicators of environmental disturbances and conditions; air and heavy metal pollution, agricultural toxins, assessing forest continuity and drought tolerance. Lichenological studies in Africa, particularly in Tanzania, have been scarce, and those available have been mainly based on morphology and chemistry data. The aim of my doctorate was to investigate lichens, in particular *Usnea* and calicioid lichens in mountain rain forests in Tanzania, using both traditional and molecular approaches.

Paper I and **II** explored *Usnea* subgenus *Eumitria*. In **paper I**, molecular, morphological and chemical methods were utilized. A phylogeny of *Eumitria* from Tanzania based on a four-markers data set supported monophyly of *Eumitria*, where sixty-two new sequences were reported. In **paper II** additional specimens of the *Usnea pectinata* aggregate from Tanzania and São Tomé and Príncipe were studied, and forty-two specimens were examined by an integrative approach (molecular, morphological, chemical data). The *U. pectinata* aggregate was monophyletic, containing several subclades, some characterized morphologically and chemically.

Paper III and **IV** focused on calicioids. **Paper III** summed up earlier information on Tanzanian calicioids along with new discoveries (twenty-six species), with notes on their habitats and distributions. *Chaenothecopsis kilimanjaroensis* was described as new, *Chaenotheca hispidula* and *Pyrgillus cambodiensis* new to Africa: *Calicium lenticulare* and *Chaenothecopsis debilis* new to Tanzania. In **paper IV**, *Coniocybe* was revised and emended to include along with its type *C. furfuracea*, also *C. brachypoda* and *C. confusa*. A three marker phylogeny was used to infer its phylogenetic position and *Coniocybe eufuracea* was described as new.

This thesis contributes to the knowledge of the lichens in Tanzania and Africa at large by building capacity in lichenology and its applications for future research. It provided integrated data for *Usnea* species from Africa, adding to the knowledge of this difficult group (only two sequences of *Usnea* from Africa have previously been published). It provided new information on calicioid lichens in Tanzania and by uncovering a rich diversity in both of the groups studied provided a foundation for further investigations of lichen biodiversity.

Keywords: Lichens, *Usnea*, calicioids, molecular phylogeny, secondary chemistry, morphotypes

Stella Gilbert Temu, Department of Organismal Biology, Systematic Biology, Norbyv. 18 D, Uppsala University, SE-75236 Uppsala, Sweden.

© Stella Gilbert Temu 2020

ISSN 1651-6214

ISBN 978-91-513-1043-5

urn:nbn:se:uu:diva-422950 (<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-422950>)

To my father

Cover: *Usnea baileyi* (Stirt.) Zahlbr., photo by Phillipe Clerc; *Coniocybe eufuracea* Temu & Tibell sp. nov., photo by George Hillman; edited by Polycarp Laurent.

List of Papers

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

- I. **Temu, S.G.**, Clerc, P., Tibell, L., Tibuhwa, D.D., Tibell, S. (2019). Phylogeny of the Subgenus *Eumitria* in Tanzania. *Mycology* 10: 250–260.
<https://doi.org/10.1080/21501203.2019.1635217>.
- II. **Temu, S.G.**, Clerc, P., Nadel R.A.M., Tibell, L., Tibuhwa, D.D., Tibell, S. The *Usnea pectinata* aggregate, Molecular, Morphological and Chemical Variation. *Manuscript*.
- III. **Temu, S.G.**, Tibell, S., Tibuhwa, D.D., Tibell, L. (2019). Crustose Calicioid Lichens and Fungi in Mountain Cloud Forests of Tanzania. *Microorganisms* 7:491.
<https://doi.org/10.3390/microorganisms7110491>.
- IV. **Temu, S.G.**, Tibell, S., Tibuhwa, D.D., Tibell, L. *Coniocybe* Ach., Revision of a Genus of Calicioid Lichens. *Manuscript*.

Important note: Paper IV of this thesis is a manuscript that contains nomenclatural novelties. In order to make it clear that these are not validly published in this thesis the basionym necessary according to the International Code of Botanical Nomenclature was omitted and a holotype is not indicated.

My contributions to the papers

The contributions of Stella Gilbert Temu to the papers included in this thesis were as follows:

In all the papers included in this thesis I have made primary contribution to the experimental design, specimen collection, data generation and analyses, and manuscript preparation as detailed below;

I: Main author. I participated in the collection of specimens and their morphological characterization, performed all the molecular and chemical experiments, performed all data analyses, wrote a first draft of the manuscript and contributed to editing the final version.

II: Main author. I participated in the collection of Tanzanian specimens and their morphological characterization, performed all the molecular and chemical experiments for Tanzanian specimens and part of the São Tomé and Príncipe specimens (nuLSU), performed all analyses, wrote a first draft of the manuscript and contributed to editing the final version.

III: Main author. I participated in the collection of specimens, carried out all molecular experiments, participated in their morphological characterization, performed all data analysis, wrote a first draft of the manuscript and contributed to edit the final version.

IV: Main author. I participated in the collection of specimens, carried out all molecular experiments, participated in their morphological characterization, performed all data analysis, wrote a first draft of the manuscript and contributed to editing the final version.

Contents

Introduction	11
Lichens.....	11
Why study lichens in Tanzania?	11
Lichenology in Tanzania.....	12
Tropical mountain forests.....	13
The genus <i>Usnea</i> (Parmeliaceae)	13
Infrageneric clades within <i>Usnea</i>	15
The calicioids.....	16
Thesis Aims.....	19
Specific aim of each paper.....	19
Comments on the methods	20
Study sites.....	20
Morphological and anatomical studies of <i>Usnea</i>	21
Morphological studies of calicioids lichens	22
Chemical studies.....	22
DNA extraction, PCR amplifications and sequencing	22
Phylogenetic analyses.....	23
Summaries of papers	24
Paper I: Phylogeny of the subgenus <i>Eumitria</i> in Tanzania	24
Paper II: The <i>Usnea pectinata</i> aggregate, molecular, morphological and chemical variation.....	26
Paper III: Crustose calicioid lichens and fungi in mountain cloud forests of Tanzania.....	29
Paper IV: <i>Coniocybe</i> Ach., revision of a genus of calicioid lichens	31
Concluding remarks and future prospects.....	34
Svensk sammanfattning.....	36
Acknowledgements	38
References	42

Abbreviations

AIC	Akaike Information Criterion
DNA	Deoxyribonucleic acid
ITS	Internal Transcribed Spacer
KOH	Potassium hydroxide
MCM7	Minichromosome Maintenance Component 7
MLbs	Maximum Likelihood bootstrap support
PP	Posterior Probability
LSU	Large ribosomal subunit
PCR	Polymerase Chain Reaction
RPB1	RNA Polymerase II first-largest subunit
SEM	Scanning Electron Microscope
s. lat.	sensu lato (in a broad sense)
s. str.	sensu stricto (in a narrow sense)
TLC	Thin Layer Chromatography

Introduction

Lichen studies on the African continent have been rather few and as a consequence there are limited data on species diversity and distribution. Molecular data are scanty. I focused on studying lichens so as to contribute to the knowledge of them in Africa, particularly in Tanzania. Lichens grow in various habitats in Tanzania, and are often prominent in undisturbed, humid forests.

In the following sections I will provide an introduction to the lichens with special reference to the 'beard lichens' (*Usnea* Adans., Parmeliaceae) and the calicioid lichens.

Lichens

'Consider the Lichen. Lichens are just about the hardiest visible organisms on Earth, but the least ambitious.' (Bill Bryson, 2003)

Lichens are fungi living in a symbiotic relationship with microscopic green algae or cyanobacteria, the symbiotic partner providing carbohydrates to the symbiosis (Honegger 1998). In recent years, non-photosynthesising bacteria (Grube et al. 2015) have been found to influence the symbiosis (Aschenbrenner et al. 2014; Wedin et al. 2016). Basidiomycetes have also been found to often be part of lichen symbioses (Spribille et al. 2016; Tuovinen et al. 2019). Lichens vary in growth forms, from crustose, to foliose (leaf like) or fruticose (shrubby or bush-like). Lichens occur in a variety of environments and on different substrates such as rocks, soil, wood, and on the bark of trees (Seymour et al. 2005). The species often have wide geographical distributions.

Why study lichens in Tanzania?

My PhD project aimed at building research capacity in mycological sciences in Tanzania, and more specifically in the field of lichenology since this, so far, has been missing in the country. In spite of having mountain forests with an extremely rich and varied lichen biota our scientific knowledge about them remains scanty. In my PhD studies, I have been exploring the genus *Usnea*,

the so called ‘beard lichens’, and calicioid lichens and fungi, the ‘pin-lichens’, which have derived their names from the fact that their fruit-bodies look like small nails. Both these groups have figured prominently when lichens have been used as bioindicators assessing e.g. air quality and in forest ecology and history. *Usnea* species are often quite spectacular and have frequently been noticed and collected even by non-lichenologists and are also well-known and often used among indigenous people for various domestic usage, not only as food but also as drugs, for dyeing and various other purposes. *Usnea* is widely known and utilized in medical treatments. It exhibits a wide range of medical effects i. a. due to the presence of usnic acid, a potent antibiotic. Applications have included antiviral, and antibacterial treatment of wounds and burns (Podterob 2008). Calicioids are important as bioindicators, and in nature conservation indicate species rich areas and habitats with long forest continuity (Tibell 1992). Studying the systematics of lichens will contribute to an increased knowledge of the lichens diversity of Tanzania and also in Africa in general. Systematics studies of lichens in Tanzania have been few and mainly base on traditionally methods – morphology and secondary chemistry, whereas molecular information has been scarce or lacking. Deforestation and climate change are among major threats to biodiversity in Tanzania. They both affect the mountain forests and their rich lichen diversity. Local scientific lichenological competence is needed for successful guidance of conservation measures in areas and habitats important for lichen diversity and moreover also general biodiversity. This thesis is a further step towards deepening the knowledge of lichens in mountain forests in Tanzania and Africa at large. It provides baseline data on *Usnea* and calicioids, and adds new molecular information. Thus it provides a basis for further exploration of the biodiversity and potential utilization of lichens in this part of the world.

Lichenology in Tanzania

The knowledge of Tanzanian lichens is rather limited and based on few published scientific investigations. As in many other areas macrolichens have obtained more attention than the crustose lichens. Access to molecular information is very scant. A main reason is the lack of local lichenological competence in Tanzania. Most studies have been carried out by visiting scientists from other countries, for example in the Kili project (Kaasalainen et al. 2018). Several lichens collected in Tanzania, including some *Usnea* species, were distributed by Vězda (2008) in his exsiccata.

Major contributions to the Tanzanian lichen flora were provided by T.D.V. Swinscow and H. Krog in treatises of East African lichens, which, however, focused on macrolichens (Krog 1993, 1994, Krog & Swinscow 1982, 1983, Swinscow & Krog 1986a, 1986b, 1986c). They also published comprehensive treatments of the macrolichens of East Africa (Swinscow & Krog 1988) and on corticolous macrolichens of low montane forests (Krog 2000). In the

beginning of 21st century V. Alstrup made important contributions to the knowledge of the lichen flora in Tanzania (Alstrup & Aptroot 2005; Alstrup & Christensen 2006; Alstrup et al. 2010).

None of the above studies included molecular information. Given the importance of molecular data in modern lichen systematics, there is a great need to extend molecular investigations of lichens collected in Tanzania.

Tropical mountain forests

Tropical mountain forests harbour important biodiversity. They are, however, threatened by the human impact such as felling of tropical forests for agriculture; overgrazing; timber production; charcoal burning and bush fires (FAO 2010a). This threatens the biodiversity of mountain forest biodiversity. Most tropical forests are thus disturbed by the increased logging, fires and forest plantation (Gibson et al. 2011). Few undisturbed tropical forests exist today, and their unique ecology is further threatened by human driven factors and climate change that have impact on the temperature, rainfall as well as the formation of clouds in mountain areas (Bubb et al. 2004). The precise contribution of these factors on the structure and composition or disappearances of the montane forests remain unknown. A report from the Global Forest Resources Assessment 2010 documented that about 16 million hectares of global forests were converted or lost by natural causes each year in 1990–2000 (FAO 2010b).

About 38 % of the total land area in mainland Tanzania is forest biodiversity conservation areas (33.5 million hectares, Lambrecht et al. 2002). These are important in order to safeguard endemic species and also for climatic and land care reasons (Bubb et al. 2004; Benítez et al. 2012).

The Kilimanjaro National park forest comprises several altitudinal zones; the colline (lowland) zone; the submontane zone; the lower (elevation 1800–2200 m), the middle (2200–2500 m), and upper (2500–3850 m) montane zones, and the subalpine forest zone (4000 m and above) (Hemp 2006a; 2006b).

The genus *Usnea* (Parmeliaceae)

Usnea is one of the largest genera in the family Parmeliaceae. It comprises about 350 species (Divakar et al. 2015; Thell et al. 2012). In the phylogeny of Parmeliaceae, *Usnea* forms a strongly supported monophyletic clade, in the ‘usneoid’ clade (Divakar et al. 2015; Crespo et al. 2007). *Usnea* includes fruticose lichen-forming fungi described as having a beard-like thallus (Figure 1).



Figure 1. Stella Temu and Prof. Donatha D. Tibuhwa from University Dar es Salaam collecting *Usnea* on Mount Kilimanjaro. Photo by Frank Mbago.

The genus is characterized by having radially symmetric branches with a central axis consisting of an elastic, cartilaginous strand of longitudinally orientated hyphae and by containing usnic acid in the cortex (Wirtz et al. 2006). *Usnea* is known for the morphological variability of its species, which has caused difficulties in species recognition and characterization. A major work about *Usnea* was published in the 20th century by Josef Motyka (1936, 1938). More than 750 names were published in this world monograph. His work was challenged as being based on morphological characters where a species sometimes might differ from another by just one character, characters that, it could be argued, perhaps were modified by environmental factors (Clerc 1998). Furthermore, Motyka's fieldwork was carried out mainly in Eastern Europe and most of the tropical species he described were based on herbarium material (Clerc 1998).

In Motyka's time the studies of lichen chemistry were at an early stage. Only subsequently, and particularly after the advent of thin layer chromatography (TLC), secondary chemistry of lichens could with precision be studied in detail. Such information was included regarding East African material in Swinscow & Krog (1976a-b). Studies of the genus was carried out in different

places: Africa (Swinscow & Krog 1978, 1979, 1988), Argentina (Rodriguez 2011, Rodriguez et al. 2011), Australia (Stevens 1992, 1999, 2004), Europe (Clerc 1984, 1987a-b, 1992, 1994, 2006, 2011; Halonen et al. 1998, 1999; Fos & Clerc 2000; Caviro 2015), India (Awasthi 1986), Japan and Taiwan (Ohmura 2001, 2012), New Zealand (Galloway 2007), North America (Tavares & Sanders 1998; Herrera-Campos et al. 1998, 2001; Clerc 2007; Hinds & Hinds 2007; Herrera-Campos 2016), the Polar regions (Walker 1985; Wirtz et al. 2008, 2012), and Russia (Ohmura et al. 2017). South America (Truong 2012, Truong et al. 2011, 2013a, 2013b; Truong & Clerc 2012, 2013, 2016; Gerlach et al. 2019). The genus occurs in polar, temperate and tropical regions and its center of distribution seems to be in the Neotropics (Clerc 2016). The studies reported above were based mainly on traditional methods and there are very little molecular data on the genus *Usnea* from Tanzania and Africa in general.

Infrageneric clades within *Usnea*

In a world monograph of *Usnea* by Motyka (1936, 1938), where all fruticose lichens with an inner, cartilaginous tissue were included, *Usnea* was divided into six subgenera; *Chlorea* Nyl., *Eumitria* Stirt., *Eu-Usnea* (*Usnea* s. str.), *Lethariella* Motyka, *Neuropogon* Nees & Flot. and *Protousnea* Motyka. According to Divakar et al. (2015) *Protousnea* and *Lethariella* do not belong to *Usnea*. Molecular data has revealed that *Usnea* forms a strong monophyletic group with several infrageneric clades, viz. *Dolichousnea* (Y. Ohmura) Articus, *Eumitria* and *Usnea* (Truong et al. 2013a; Temu et al. 2019a).

Eumitria, described in 1882 by Stirton, is characterized by having a fistulose central axis. However, it is similar to *Usnea* s. str. in all other major morphological and anatomical characters. *Eumitria* is known to occur in Africa (Swinscow & Krog 1974; Krog 1994; Temu et al. 2019a), Australia (Stevens 1999), Asia (Ohmura 2001, 2012) and South America (Truong & Clerc 2013). It was later considered a subgenus of *Usnea* (Motyka 1936; Ohmura 2001, 2002; Truong & Clerc 2013). *Eumitria* was, however, resurrected to generic level by Articus (2004), on the basis of molecular data, and this was also accepted by Divakar et al. (2017). It has also been shown that some species without a fistulose axis, e.g. *Usnea pectinata*, form a strong monophyletic clade with *Eumitria* (Truong et al. 2013a; Temu et al. 2019a). *Eumitria* has been considered a subgenus by several authors (Ohmura 2002; Ohmura & Kanda 2004; Wirtz et al. 2006; Temu et al. 2019a). In Divakar et al. (2017), *Eumitria* was accepted as a genus since the diversification of *Eumitria* predates the diversification of *Usnea*. This is in contrast to Thell et al. (2018) who considered the segregation of *Eumitria* as a separate genus unnecessary because of its characteristic *Usnea* morphology, i.e. possessing a central cord.

Neuropogon is characterized by the black pigmentation of the cortex, the dark pigmented apothecial discs, and a sphacelata-type cortex (Ohmura & Kanda 2004). The distribution is restricted to Antarctica, the Arctic and high Andean regions; it only occurs on rocks (Truong et al. 2013a). There are several studies using molecular data in this group (Articus 2004; Ohmura & Kanda 2004; Wirtz et al. 2006; Lumbsch & Wirtz 2011; Truong et al. 2013a; Temu et al. 2019a). However more molecular data are needed since there are indications that *Neuropogon* is polyphyletic and nested within *Usnea* s. str. (Wirtz et al. 2006; Truong et al. 2013a). The black pigmentation which characterizes this group might have evolved independently under similar ecological conditions (Wirtz et al. 2006).

Dolichousnea was segregated from *Usnea* as a subgenus based on morphological characters. It is characterized by the presence of annular pseudocyphellae, a thick hypothecium and a positive iodine reaction of the central axis (Ohmura 2001). It occurs mainly in the Northern hemisphere and includes three species (*U. diffracta* Vain., *U. longissima* Ach. and *U. trichodeoides* Vain.). They form a well-supported monophyletic clade (Ohmura 2002; Truong et al. 2013a; Temu et al. 2019a). *Dolichousnea* was elevated to a generic level by Articus (2004). Later, Divakar et al. (2017), supported these results based on an estimated time of divergence as compared to *Usnea* s. str.; Thell et al. (2018) differed with this view and suggested *Dolichousnea* to remain a subgenus within *Usnea* because of its morphological similarity to *Usnea*, viz. the central cord.

In the first part of the thesis only *Eumitria* was studied using molecular, morphological and chemical methods.

The calicioids

Calicioid lichens, also known as ‘pin lichens’, is a historical concept in which these lichens for a long period of time were considered to form a natural group, and often even a prime example of a natural group. Their recognition was mainly based on morphological characteristics such as frequently having stalked apothecia (Figure 2A), the occurrence of a mazaedium (a collection of ripe spores on the surface of the fruitbody; Figure 2B) as a result of the presence of prototunicate asci (i.e. asci from which the spores are not discharged forcefully into the air).

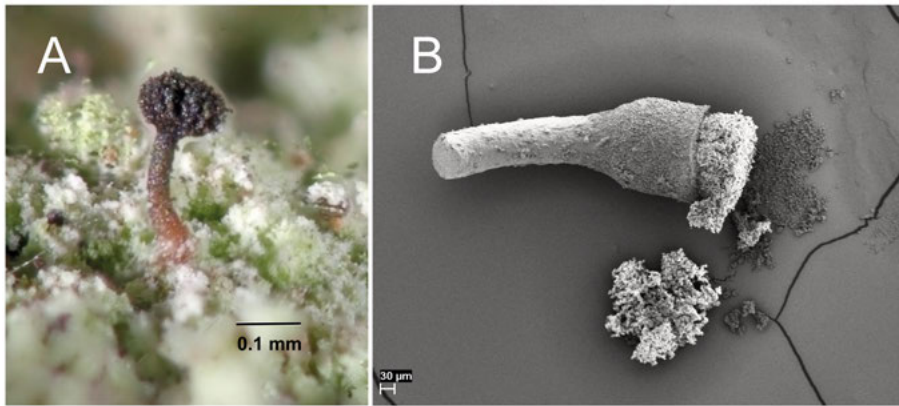


Figure 2. **A:** stalked apothecia of *Chaenothecopsis kilimanjaroensis*, photo by George Hillman; **B:** mazaedium of *Calicium hyperelloides* as visualised on SEM

Spore ornamentations are common in calicioids (Figure 3A – C). Calicioids were first given a detailed description by Acharius (1815, 1816, 1817), and they were also later to be considered as a natural group, often ranked as the order Caliciales (Bessey 1907). Keissler (1938) codified this view as ‘Coniocarpinae’. Some genera like *Chaenothecopsis* Vain. and *Stenocybe* Nyl. ex Körb. were included in ‘Coniocarpinae’/Caliciales based on a general morphological similarity with ‘core’ calicioids, viz. the presence of stalked apothecia (most of these genera were later transferred to Mycocaliciales, Tibell & Wedin 2000). Caliciales was suggested not to be a monophyletic group by Tibell (1984). This was based on morphological, chemical and ultrastructural evidence, and later supported by molecular data (Prieto et al. 2013; Prieto & Wedin 2017). Calicioids have evolved in parallel in several different clades among the ascomycetes; Arthoniales, Lecanorales, and Pyrenulales (Prieto et al. 2013). ‘Calicioids’ as used here is a concept shaped in a research tradition. It actually very precisely corresponds to Acharius’ ‘Calicioidea’ (1815, 1816, 1817). The majority of the calicioids are lichenized but some genera, like *Chaenothecopsis*, are not.

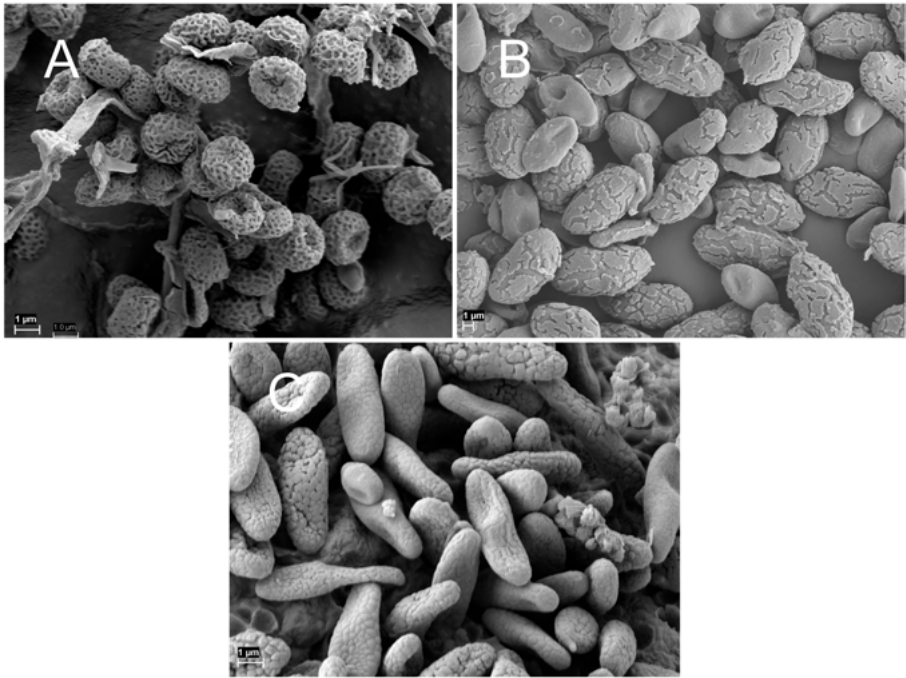


Figure 3. Different spore ornamentations among different species of calicioid lichens and fungi as visualised on SEM, **A:** *Chaenotheca furfuracea*; **B:** *Calicium hyperelloides*; **C:** *Chaenothecopsis kilimanjaroensis*

Calicioids often have wide distributions and have been studied in many parts of the world (Tibell & Wedin 2000; Titov 2000, 2001; Tibell 1987, 1996, 1998, 2001; Tibell & Thor 2003). Calicioids have been in focus in biodiversity and conservation since they are often rare and found in habitats of high conservation values (Tibell 2003). Thus they have been used as bioindicators, ‘signal species’ (Nitare 2000), for identifying such areas and also for assessing forest continuity (Tibell 1992). A high proportion of the species are on Red Lists (Kruys & Jonsson 1997; SLU Artdatabanken 2020).

In areas between the Tropic of Cancer and the Tropic of Capricorn, however, they have been much less well studied, although they often do occur in abundance at high altitudes both in Africa (Tibell 2001), Asia (Titov 2000, 2001; Tibell & Thor 2003; Tibell 2006) and in tropical South America (Tibell 1996). These occurrences offer opportunities for interesting biogeographical research and hypotheses as they harbour quite a rich and unexplored diversity of calicioids that to a large extent still remains unexplored.

Thesis Aims

The overall aim of this work was to study lichens from Tanzania with special reference to *Usnea* (Parmeliaceae) and calicioids. The general focus was to study the taxonomy of these lichens by molecular methods in addition to morphology and chemistry.

Specific aim of each paper

Paper I aimed at studying *Usnea* species of the subgenus *Eumitria* in Tanzania using a molecular approach, in addition to morphology and chemistry.

In **Paper II**, the aim was to study the *Usnea pectinata* aggregate in detail by including specimens from Tanzania and São Tomé and Príncipe; study their variation in morphology and chemistry and to compare with molecular data.

The focus of **Paper III** was to study calicioid lichens and fungi in Tanzanian cloud forests; summarize earlier information on their occurrence, to supply new data and to offer observations on their habitat and distribution.

Paper IV addressed the systematics of the revised genus *Coniocybe*. The aim of this paper is to study *Chaenotheca* s. lat., and based on molecular data emend *Coniocybe* on generic level.

Comments on the methods

Study sites

Tanzania is an East African country, and it borders the Indian Ocean to the East. Tanzania has many mountainous areas, among them Mount Kilimanjaro (5895 m), Africa's highest mountain (Figure 4). Mountain areas in Tanzania have been explored by lichenologists since the 19th century.

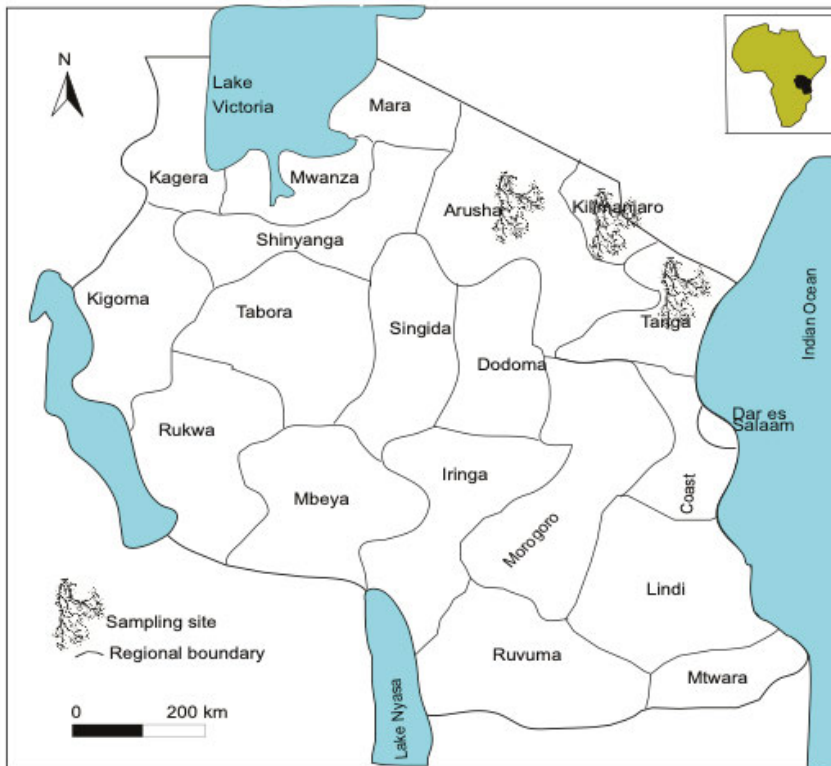


Figure 4. A map showing study sites and the regions of Tanzania. Modified from Temu et al (2019a).

Mountain cloud forests are home to a very high biodiversity (Wagner & Lugazo 2011). The climate, weather conditions, and the low level of air pollution offer good growth conditions for lichens. The elevation and the effect

of winds from the Indian Ocean provide cloud mist (Figure 5A) and frequent precipitation respectively in the mountain forests (Myers et al. 2000). The forests are well conserved despite the local population carrying out crop farming and livestock keeping nearby (Wagner & Lugazo 2011). Long-term threats against the mountain forest ecosystems are posed by an increasing population pressure and climate change.

The present studies were carried out in the northern part of the Eastern Arc Mountains (Mount Meru, Kilimanjaro, Pare and the Usambara mountains). They focused on rather undisturbed forests in four areas in the northern part of Tanzania (Figure 4), Marangu (Kilimanjaro), Monduli forest reserve (Arusha), Korogwe forests (Tanga) and the Meru forest reserve (Arusha). In some of these forests e.g. the Monduli forest reserve wild animals like buffalo and elephant occur, necessitating for field collecting being protected by guides with guns (Figure 5B) to protect the researchers.



Figure 5. Mountain forests visited in Tanzania, A: Cloud forest in Mount Kilimanjaro forest reserve; B: Stella Temu and the guides at Monduli forest reserve. Photo by Sanja Tibell.

Morphological and anatomical studies of *Usnea*

The morphology of specimens was investigated using a stereo microscope. For each specimen, three measurements were recorded: cortex (C) and medulla (M) thickness, and central axis (A) diameter. These were made on longitudinal sections of branches at $\times 50$ magnification. The relative thickness of cortex/medulla/axis of the total branch diameter (CMA) and the ratio of axis/medulla (A/M) of all the studied specimens were calculated according to Clerc (1984, 1987a) and referred to the categories defined by Clerc (2011).

Observations of the anatomical structure of the cortex were made on thin hand-cut sections at $\times 50$ as in Ohmura (2001).

Morphological studies of calicioids lichens

The morphological study of the thallus and apothecia was performed on freezing microtome sections c. 10-15 μm thick and in addition on squash preparations (ascus and spore measurements). The apothecium height, capitulum and stalk diameters were measured under a stereo microscope. The length and width of asci, and length and width of spores were measured under the light microscope and standard deviations and means were calculated. The sections were mounted in water. The spore ornamentation was observed in Scanning Electron Microscope (SEM).

Chemical studies

Chemical analyses of *Usnea* specimens were performed by thin layer chromatography (TLC) following Culberson & Ammann (1979), with solvent system B modified according to Culberson & Johnson (1982). Spot tests (mainly by KOH) were also carried out.

DNA extraction, PCR amplifications and sequencing

Total DNA was extracted from freshly collected material kept at $-20\text{ }^{\circ}\text{C}$ for less than three months using the DNeasy Plant Mini Kit (Quiagen, Hilden, Germany), following the manufacturer's instructions. Material for extraction was selected carefully to avoid contamination. For *Usnea*, a piece of a branch about 1 cm long was used while for calicioids, about 10 – 30 apothecia were used. The lichen tissue is made up of solid cell walls that must be disrupted to successful extract DNA. Thus prior to any DNA isolations, samples were prepared by freezing them in a $-80\text{ }^{\circ}\text{C}$ freezer. The frozen samples were then mechanically crushed with a tissue- lyzer machine at a speed of 25 rpm for 1 or 2 minutes.

Total DNA was used for PCR amplifications. The primers used in PCR amplifications were ITS1F (Gardes & Bruns 1993), ITS4 (White et al. 1990), LROR and LR5 (Vilgalys & Hester 1990), MCM7-709 and MCM7-1349 (Schmitt et al. 2009), gRPB1-A and gRPB1-C (Matheny et al. 2002) according to the respective study. The amplifications were done by the AccuPowerPCR PreMix (Bioneer, Daejeon, Korea); 3 μl diluted DNA, 1.5 μl of each primer (10 mM), and water was added to the premix yielding a total volume of 20 μl . The PCR conditions were specified in each study. PCR products were

visualized by electrophoresis on 1.5% agarose gels. Products were purified using Illustra™ ExoStar buffer diluted 10 x, following the manufacturer's protocol. Sequencing was carried out by Macrogen (www.macrogen.com).

Phylogenetic analyses

DNA sequences downloaded from GenBank were, along with the newly produced sequences, assembled and edited using AliView (available online: <https://ormbunkar.se/aliview/>; Larsson 2014). The newly generated sequences from each studies were aligned, along with the selected sequences of the studied group as downloaded from GenBank, by using MAFFT v.7 (available online: <https://mafft.cbrc.jp/alignment/server/>).

Phylogenetic relationships and their posterior probabilities (PP) were inferred using a Bayesian approach, and additional support values were estimated using Maximum Likelihood bootstrap support (MLbs). For the Bayesian analyses, the most likely models of evolution were estimated using the Akaike Information Criterion (AIC) as implemented in Modeltest 3.7 (Posada & Crandall 1998).

The Bayesian analysis was executed using MrBayes 3.2.6 (Ronquist et al. 2012), where two analyses of two parallel runs were carried out for 10 M generations. Each run included four chains, and trees were sampled every 1000 generations and 25% were discarded as burn in. All runs converged on the same average likelihood score and topology. Maximum likelihood estimates were carried out by RAxML version 8.2.10 using the GTR + G + I model of site substitution (Stamatakis 2014). The branch support was acquired by Maximum Likelihood bootstrapping (MLbs) of 1000 replicates (Hills & Bull 1993), and MLbs $\geq 70\%$ were considered to be significant.

The trees were visualized in FigTree version 1.3.1 (Rambaut & Drummond 2010) and edited by Adobe Illustrator.

Summaries of papers

Paper I: Phylogeny of the subgenus *Eumitria* in Tanzania

In this study, the subgenus *Eumitria* of the genus *Usnea* was investigated. The *Eumitria* specimens were collected in Tanzania. The species of this subgenus are recognized by having a fistulose axis (Figure 6D). Identification of the species in this group is difficult due to their morphological variability. Two species, *Usnea baileyi* and *U. pectinata*, were investigated (Figures 6 and 7, respectively).



Figure 6. *Usnea baileyi*, **A**: *Usnea baileyi* studied specimen (SGT 157); **B**: blackish base; **C**: soralia with short isidiomorphs; **D**: thin and shiny cortex, red subcortical pigment and tubular axis filled with loose hyphae (Temu et al. 2019a).

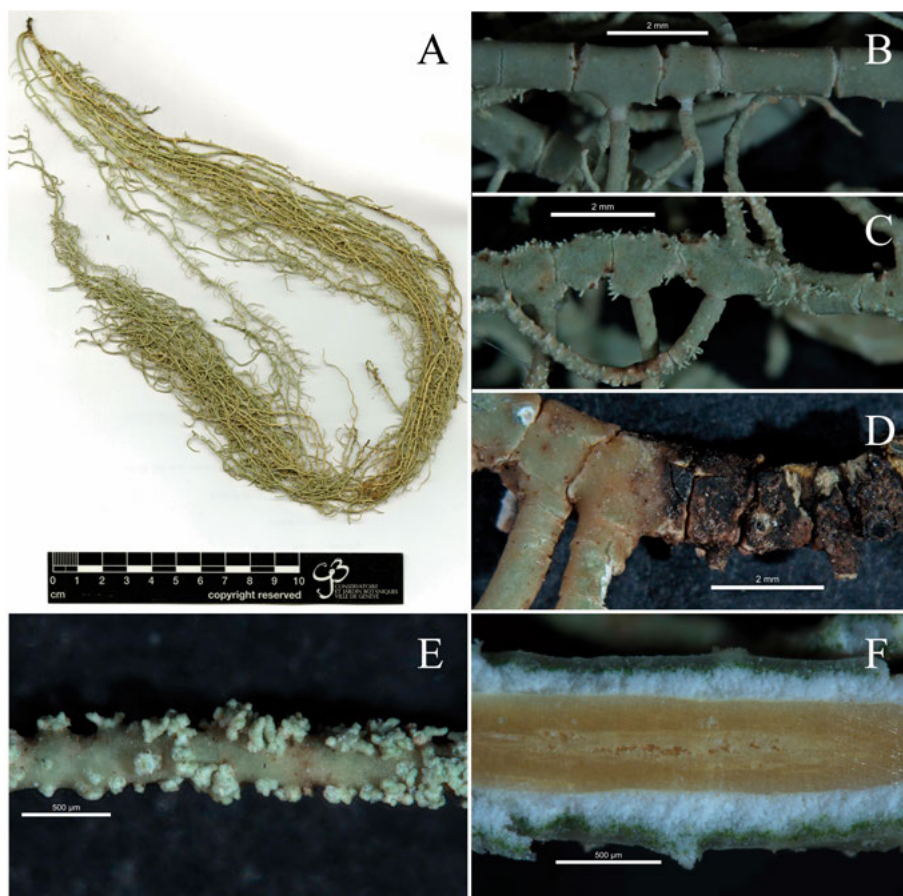


Figure 7. *Usnea pectinata*, **A**: *Usnea pectinata* studied specimen (SGT 114); **B**: main branch cylindrical with terete segments; **C**: main branch irregular with alate segments; **D**: blackish base; **E**: soralia with short isidiomorphs; **F**: dark brown pigmented axis of main branch with some fistulose areas in the central part of the axis (Temu et al. 2019a).

Morphological studies were focused on features identified by Clerc (2011) as useful for species recognition in this group. TLC was used for identifying the secondary chemical compounds. Molecular studies supplied crucial data in this group, where very few sequences were earlier available in GenBank (three ITS sequences). The phylogeny of *Eumitria* in Tanzania was presented based on molecular data and compared to morphological and chemical features (Figure 8). A total of 62 new sequences (26 ITS, 20 nuLSU, 6 MCM7, 10 RPB1) were generated. Phylogenetic analyses based on individual and

concatenated data sets of ITS, nuLSU, MCM7 and RPB1 were used to infer the phylogeny of *Eumitria*. Molecular analyses showed strongly supported monophyletic clades of *U. baileyi* and *U. pectinata*, respectively. Morphological features such as the pigmentation of the axis, branch shape, and chemical patterns showed infraspecific variation in *U. pectinata* (Figure 8).

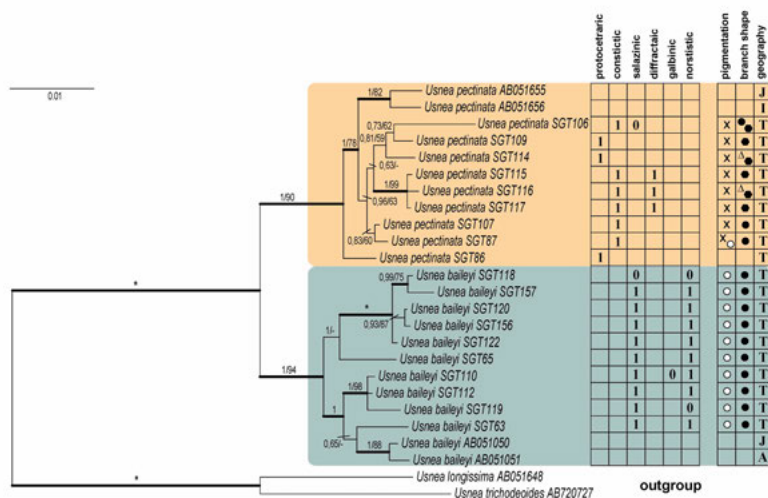


Figure 8. Consensus tree based on Bayesian and ML analyses of *Eumitria* species in Tanzania (ITS, nuLSU, RPB1 & MCM7). The two support values associated with each internal branch correspond to posterior probability (PP) and bootstrap support (bs) respectively. Branches in bold indicate a support of PP $\geq 95\%$ and a MLbs $\geq 70\%$. An asterisk on a bold branch indicates that this node has a support of 100% for both support estimates. A dash instead of MLbs value indicates that the node of the Bayesian tree was not recovered by ML bootstrapping. A: America, I: Indonesia, J: Japan, T: Tanzania 1: main chemical substance, 0 accessory chemical substance, x: dark brown pigmentation, big black dots: terete branch shape, triangles: alate branch shape, pentagon: ridged branch shape (Temu et al. 2019a).

Paper II: The *Usnea pectinata* aggregate, molecular, morphological and chemical variation

Usnea pectinata Taylor is characterized by having a pendent thallus with elongated terminal branches, a dark brown base, punctiform maculae on lateral branches and the presence of stictic acid as a major substance (Ohmura 2001). This study is a continuation of **paper I** including additional specimens of the

U. pectinata aggregate. Altogether 42 specimens were analysed, 22 from Tanzania and 20 from São Tomé and Príncipe. The variation of specimens belonging to the *U. pectinata* aggr. in the marker sequences, morphology and secondary chemistry was examined.

An ITS phylogeny was generated from the dataset that contains thirty-three (33) sequences newly produced sequences and three (3) sequences downloaded from GenBank. Apart from the sequences produced in **paper I** and the newly produced sequences in this study, there was previously only three (3) ITS sequences of *U. pectinata* s. str. available in GenBank, originating from Indonesia, Japan and Taiwan. No *U. pectinata* sequences from Africa have earlier been published. Thus, this study is the first major molecular work on the *U. pectinata* aggr.

In the ITS-tree of the *U. pectinata* aggr. morphological, chemical and geographical features were indicated (Figure 9). In a phylogeny, the monophyly of *U. pectinata* aggr. along with seven strongly supported subclades (A – G), was observed. These clades obtained high support from both Bayesian and MLbs analyses.

A total of seven chemotypes were observed (Figure 9) considering main substances as detected by TLC. Six of them were observed in the *U. pectinata* aggr. from Tanzania and São Tomé and Príncipe and one was described by Ohmura (2001). The chemotypes contained the following secondary substances: (1) protocetraric acid (2) constictic acid (3) protocetraric and constictic acid (4) salazinic and diffractaic acid (5) constictic and diffractaic acid (6) salazinic acid (7) stictic acid. However, the stictic acid group reported by Ohmura (2001) was not observed in the African material studied.

The specimens represented two and four morphotypes characterized by their axis pigmentation and branch shape, respectively (Figure 9). A pale to dark brown axis pigmentation was observed. Four distinct branch shapes have been noted; terete, ridged, alate and flattened. A correlation between chemistry, morphology and molecular data was observed in most of the well supported clades (B, C, and F; Figure 9).

The name '*U. pectinata* aggregate' is here used as a place holder of what we see as a species complex. From this point of view, our results might well be in line with those of Truong et al. (2013a), Saag et al. (2011), Ohmura (2008), Wirtz et al. (2008) who reported that *Usnea* species, as recognized by morphological and chemical characters, are usually monophyletic. More specimens from different part of the world should be studied before proceeding with taxonomical decisions on the *U. pectinata* aggr.

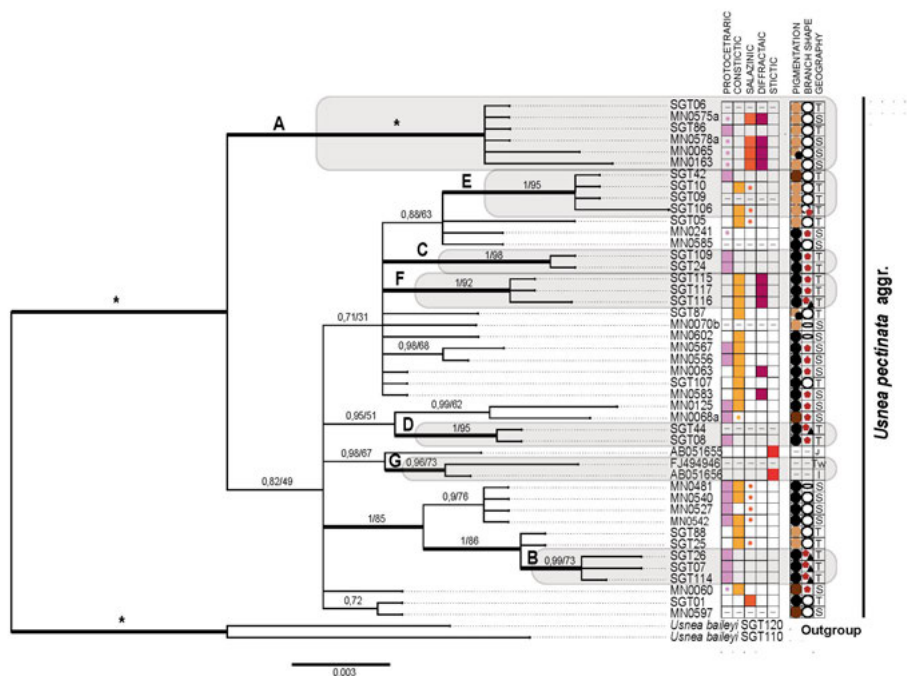


Figure 9. ITS phylogeny based on Bayesian and Maximum Likelihood (ML) analyses of the *Usnea pectinata* aggregate along with morphological and chemical data. The two support values associated with each internal branch correspond to posterior probability (PP) and bootstrap support (bs) respectively. Branches in bold indicate a support of PP $\geq 95\%$ and a MLbs $\geq 70\%$. An asterisk on a bold branch indicates that this node has maximum support for both support estimates. Full square shade: main chemical substance, small coloured dot: accessory chemical substance, dash: unknown. Usnic acid is found in all specimens and is therefore not included. Pale brown circle: pale brown pigmentation, dark brown circle: dark brown pigmentation, white circle: terete branch shape, flattened white circle: flattened branch shape, triangles: alate branch shape, pentagon: ridged branch shape. I: Indonesia, Tw: Taiwan, S: São Tomé and Príncipe, T: Tanzania.

Paper III: Crustose calicioid lichens and fungi in mountain cloud forests of Tanzania

Calicioids also known as ‘pin lichens’ have got this nickname because of their stalked fruit bodies (Figure 11A). Calicioid lichens and fungi are, however, an artificial concept and rather reflects a research tradition – but not a natural grouping. In this study the calicioids were treated in traditionally sense. Some species similar to the ‘pin-lichens’ are also included in the calicioids; in short species with a mazaedium and/or with stalked apothecia. Crustose calicioid lichens and fungi in mountain cloud forests in Tanzania were investigated. The material was collected in three different mountain forests in the northern part of Tanzania: Monduli (Arusha), Mount Meru (Arusha) and Kilimanjaro national park (Kilimanjaro). The areas are well protected and are renowned for their biodiversity. The calicioids were found to occur mainly in the upper forest zones where the common substrates were bark and decorticated stumps of trees. The morphology and chemistry (mostly spot tests with KOH) were studied. A total of 26 crustose calicioids (Figure 10) were recorded and notes on their occurrence, ecology and distribution given. *Calicium lenticulare* and *Chaenothecopsis debilis* were recorded as new to Tanzania. *Chaenotheca hispidula* and *Pyrgillus cambodiensis* were new to Africa. *Chaenothecopsis kilimanjaroensis* (Figure 11) was described as new to science based on molecular and morphological features.

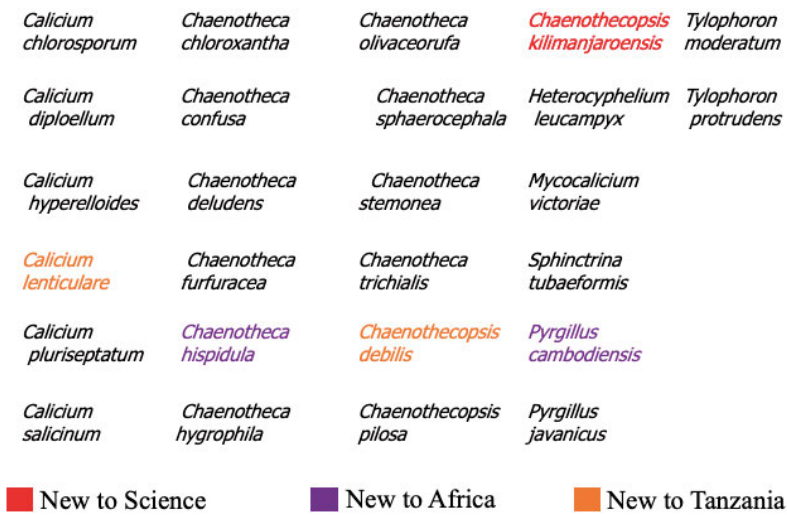


Figure 10. Calicioid lichens and fungi from mountain cloud forest of Tanzania, (Temu et al. 2019b).

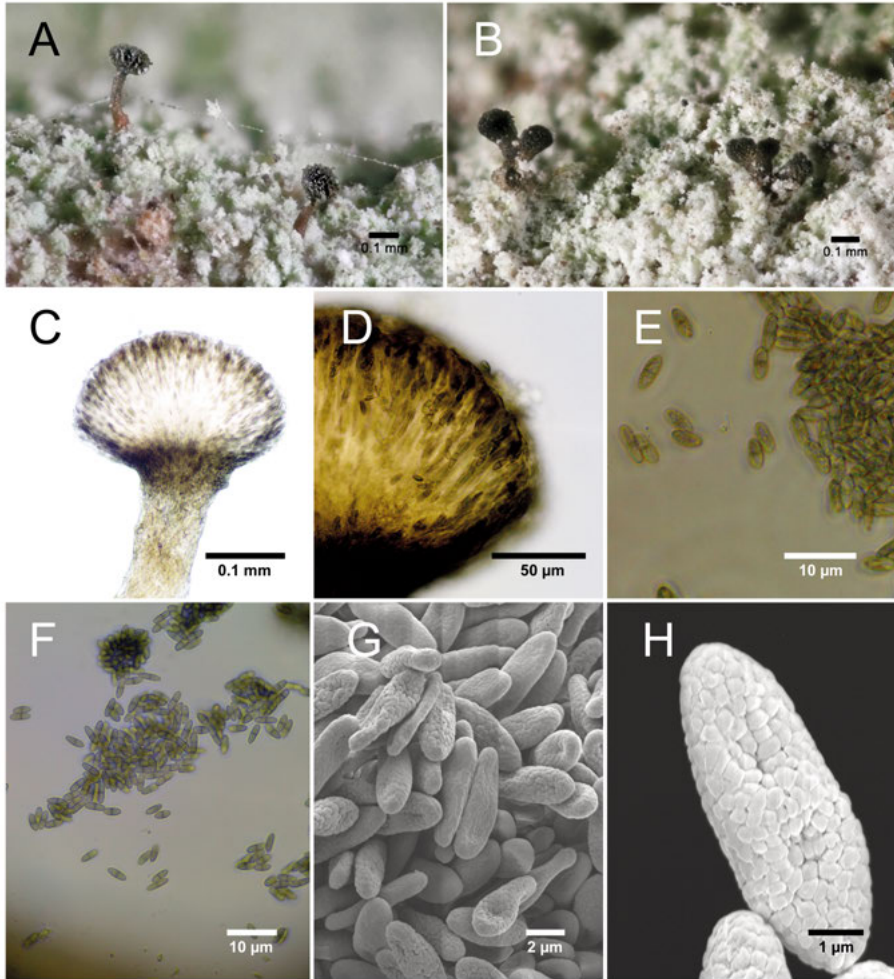


Figure 11. *Chaenothecopsis kilimanjaroensis*, **A**: Well-stalked apothecia with brown stalks; **B**: aggregated apothecia; **C**: section of apothecium with pale stalk; **D**: dark brown, well-developed excipulum; **E**: spores with a faint ornamentation as barely visible under the light microscope; **F**: spores; **G**: spores, SEM; **H**: verrucose spore ornamentation, SEM (Temu et al. 2019b).

A phylogeny of *Chaenothecopsis* was presented (Figure 12), where *Chaenothecopsis kilimanjaroensis* was found to be close to *C. debilis*.

in fact the only crustose calicioid described by Linnaeus. Further species included in *Coniocybe* by Acharius (1816) were *C. stilbea* Ach., *C. brachypoda* Ach., and *C. gracilentia* (Ach.) Ach. *Coniocybe* was later accepted by E. Fries (1831) and also by Th.M. Fries (1860, 1861) and Zahlbruckner (1926), as different from *Chaenotheca* in having a poorly developed excipulum. *Coniocybe* was maintained and widely used until Tibell (1984) transferred *Coniocybe brachypoda* and *C. furfuracea* to *Chaenotheca* based on morphology and chemistry data. Later, *Chaenotheca confusa* Tibell, which is very similar to *C. furfuracea* and here referred to *Coniocybe* was described (Tibell 1998).

In this study, specimens from different parts of the world were included. Based on molecular, morphological and chemistry data, we propose an emendation of *Coniocybe* to include *C. brachypoda*, *C. furfuracea* and *C. confusa* along with the newly described species *C. eufuracea* (Figure 13).

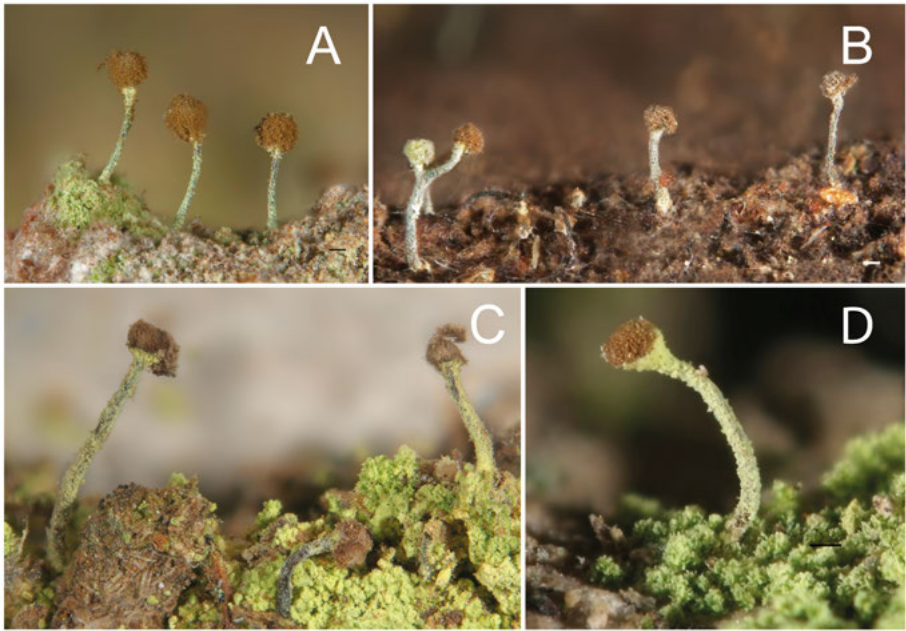


Figure 13. Ascomata of *Coniocybe* species, **A:** *C. eufuracea* (Temu 422); **B:** *C. brachypoda* (Tibell 17062); **C:** *C. confusa* (Kantvilas 280/19); **D:** *C. furfuracea* (Temu 442). Pictures by George Hillman.

Analyses of *Chaenotheca* s.lat. based on a three-marker dataset (ITS, nuLSU and RPB1) were carried out based on a wide selection of species of *Chaenotheca* s. lat. (i. e. sensu Tibell 1984) with representation of all the *Chaenotheca* subclades mentioned in Tibell et al. (2019). In the phylogeny *Coniocybe* s. str was distinct from other clades of *Chaenotheca* s. lat. and it

has maximum support in Bayesian and Maximum Likelihood analyses. In order to clarify the relationships within *Coniocybe*, further analyses of ITS were performed and the phylogeny is presented in Figure 14.

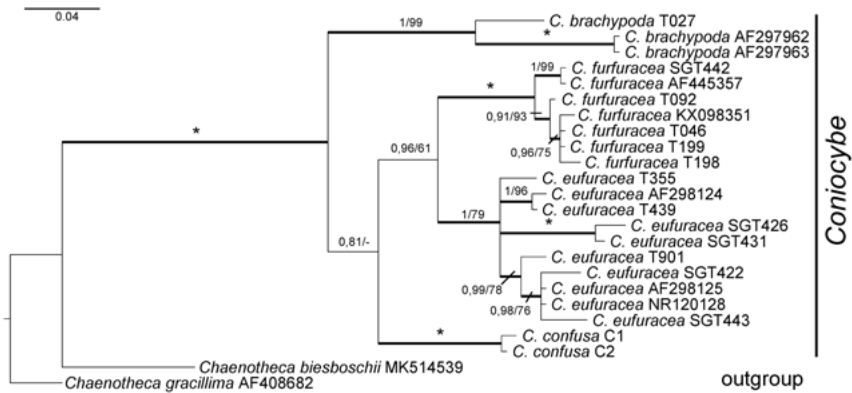


Figure 14. Phylogenetic relationships of 4 species of *Coniocybe* based on a Bayesian and Maximum Likelihood (ML) analyses of an ITS dataset. The tree was rooted using *Chaenotheca biesboschii* and *Chaenotheca gracillima*. The two support values associated with each internal branch correspond to posterior probabilities (PP) and maximum likelihood bootstrap support (MLbs) proportions, respectively. Branches in bold indicate a support of PP $\geq 95\%$ and MLbs $\geq 70\%$. An asterisk on a bold branch indicates that this node has a support of 100% for both support estimates.

Concluding remarks and future prospects

This thesis consists of four papers, which aimed at investigating lichens from Tanzanian mountain rainforests with special reference to *Usnea* (Parmeliaceae; paper I and II) and calicioids (paper III and IV), bringing an important contribution to the knowledge of African lichens. In an integrative approach, morphology along with chemical and molecular data, were used. Comments on the ecology and distribution of the species were included. Since the study involved two different groups of lichens, I will give concluding remarks for each group separately.

In **paper I** and **II**, I dealt with the fruticose genus *Usnea*, which is known for a morphoplasticity that has caused difficulties in species recognition and characterization. In **paper I**, species from *Usnea* subgenus *Eumitria* were studied. A phylogeny of *Eumitria* in Tanzania was presented. A total of 62 new sequences (26 ITS, 20 nuLSU, 6 MCM7, 10 RPB1) for *Eumitria* were produced. This brings a considerable contribution to the knowledge of *Usnea* in Tanzania and Africa, given that earlier only two (2) DNA sequences were available in GenBank. Chemical and morphology data were also provided. In **paper II**, the *Usnea pectinata* aggregate was studied in detail by including more specimens (from Tanzania and São Tomé and Príncipe) using classical and molecular approaches. The genetic variation was studied along with the morphology and chemistry of the species. These data are summarized in a phylogeny (Figure 8). Seven subclades were observed within the *Usnea pectinata* aggregate, and two and four morphotypes were found as axis pigmentation and the shape of branches, respectively, were noted. Six chemotypes were found among the studied specimens. These chemotypes have not been reported in previous studies of *U. pectinata*. The paper brought an important progress in treating the *Usnea pectinata* aggr. from Africa, for which previously molecular data worldwide was very scanty, and in total only four (4) DNA sequences were available in GenBank. These studies revealed considerable variability and ensuing complications in recognizing species in *Usnea*.

During my PhD studies I collected about 300 specimens of *Usnea* from Tanzania, whereby I also managed to study their morphology. Apart from the studied material, I also produced additionally ninety one (91) more ITS sequences for *Usnea*. I aligned these with all *Usnea* ITS sequences available in GenBank as a base for future work on *Usnea* from Tanzania.

Since *Usnea* are well known to contain bioactive compounds, I also aimed at studying bioactive compounds in *Usnea* and their activity. I started at

profiling the compounds in High-performance liquid chromatography (HPLC) and it looks promising since some bioactive compounds were found; usnic acid being one of them (results not presented in the thesis). This is an applied field worthy to pursue in future work, and further research should be directed to search for additional bioactive compounds in *Usnea* and investigating their efficacy.

Paper III and **IV** aimed at investigating calicioid lichens and fungi in mountain cloud forests in Tanzania. In **paper III** a total of 26 calicioid lichens and fungi were recorded. It contained information on new records to science, Africa and Tanzania. The paper further contained comments on the habitat and distribution of the species. **Paper IV** focused on *Coniocybe* and the genus was revised and emended to include in addition to the two species originally part of the genus (*C. furfuracea* and *C. brachypoda*), also *C. eufuracea* - a species new to science and another species (*Chaenotheca confusa*) combined into *Coniocybe*.

Surveys of lichens in mountain cloud forests are important since they harbor a high lichen diversity, and future research should also address conservation issues in order to safeguard the ecological continuity of these forests now at peril due to changing land use and climate change.

Generally, I would say that Tanzania is a country with one of Africa's richest pools of biodiversity, that still is insufficiently known. The results of this thesis have advanced the knowledge on the lichens from Africa. Most of the results form a baseline for future research on lichens in Africa. Still the lichenological studies in Africa are very limited and in the future research genetic studies will be of great importance. Research on other groups of lichens is badly needed as a basis for using lichens in bioindication.

Svensk sammanfattning

Denna avhandling behandlar taxonomin hos lavar i bergsregnskogar i Tanzania. Två grupper av lavar har studerats, släktet skägglavar *Usnea* och calicioida lavar ("knappnåslavar") med målet att generellt öka kunskapen om lavar i Afrika. Lavar bidrar med viktiga ekosystemtjänster och används ofta inom praktisk naturvård. De kan exempelvis användas som bioindikatorer i undersökningar av miljöpåverkan från t.ex. luftföroreningar, tungmetaller, jordbrukskemikalier och skogsbruk. Den biologiska mångfalden i Afrika är fortfarande dåligt studerad och det är angeläget att klarlägga denna ytterligare med avseende på lavarnas taxonomi, nomenklatur, utbredning och ekologi. Ofta används morfologi och kemi för att särskilja lavararter. Numera används också allt oftare molekylära metoder i kombination med de mer traditionella metoderna. Mycket få studier med molekylära metoder har utförts på afrikanskt material av lavar. I denna avhandling har jag studerat skägglavar *Usnea* och calicioida lavar och svampar med en kombination av traditionella och molekylära metoder.

I **studie I** använde jag en kombination av molekylära metoder, morfologi och kemi för att studera arter i *Usnea* undersläktet *Eumitria* (Parmeliaceae). Material insamlades vid fältarbete i Tanzania. Två arter studerades, *Usnea baileyi* och *U. pectinata*. Mina studier visade att medan morfologin och kemin är likformig hos *U. baileyi* så är både kemin och morfologin variabel inom *U. pectinata*. Här kunde flera kemotyper och morfotyper urskiljas. Studien resulterade i 62 nya sekvenser (26 ITS, 20 nuLSU, 6 MCM7, 10 RPB1) för *Usnea* subgenus *Eumitria*.

I **studie II** studerade jag den variabla *Usnea pectinata* aggregatet i mer detalj med molekylära metoder. Här inkluderas material både från Tanzania och São Tomé. Variationen var stor men i ett släktträd baserat på ITS delade materialet upp sig i två klader vilka dock inte gick att särskilja åt med morfologi eller kemi. Arten varierade med avseende på centralaxelns färg och grenarnas form. Således kunde fem distinkta kemotyper urskiljas som skiljer sig från de kemotyper som tidigare rapporterats för denna art. Resultaten visar den morfologiska och kemiska plasticitet som finns inom det studerade komplexet av skägglavar, samt att stora dataset omfattande såväl morfologi, kemi som sekvenser behövs för framgångsrika och detaljerade studier av detta släkte med morfologiskt variabla och följaktligen svåridentifierade arter.

I **studie III** reviderade jag taxonomin hos calicioida lavar och svampar ("knappnåslavar") i Tanzania. Studien syftade till att sammanställa tidigare

information om calicioida lavar och svampar i Tanzania och tillföra ny kunskap baserad på fältarbete i bergsregnskogar. Calicioida lavar och svampar har ofta specifika krav på sin livsmiljö och är ofta rödlistade. De kan därför användas som bioindikatorer på t.ex. lång skoglig kontinuitet. Gruppen är artrik i bergsregnskogar och ska därför uppmärksammas vid skydd av dessa miljöer liksom vid upprättandet av skötselplaner. *Chaenothecopsis kilimanjaroensis* nybeskrivs baserat på molekylära data och morfologi. *Calicium lenticulare* och *Chaenothecopsis debilis* rapporteras som nya för Tanzania medan *Chaenotheca hispidula* och *Pyrgillus cambodiensis* rapporteras som nya för Afrika. Totalt 26 skorpformade arter av calicioida lavar och svampar inkluderades. De flesta av dessa arter har vida utbredningar i kalla områden av båda hemisfärerna men har även spridda förekomster på höga berg på lägre latituder, vilket inbjuder till intressanta biogeografiska studier. De flesta av arterna förekommer främst i den mellersta och övre skogszonen och växer på bark av äldre träd och på ved.

I **studie IV** reviderar jag släktet *Coniocybe* och förutom typarten *C. furfuracea* inkluderas även *C. brachypoda*, *C. confusa* samt den nya arten *C. eufuracea*. Historiskt har ytterligare arter förts till släktet, men dessa arter ska föras till andra släkten. Resultaten visar att *Coniocybe* är monofyletiskt i en molekylär studie där ITS, nuLSU och RPB1 sekvenser använts för att klarlägga fylogenin.

Resultaten från denna avhandling utgör en gedigen grund för framtida forskning om lavar i Tanzania och övriga Afrika, särskilt avseende skägglavar *Usnea* och calicioideer, som båda tidigare endast sparsamt studerats.

Acknowledgements

As I accomplish this important academic milestone, my heart is filled with countless gratitude.

My profound gratitude goes to my academic supervisors and mentors; **Donatha Tibuhwa**; I am so proud to be your student. You are one of the unique Tanzanian female Professor and scientist I ever knew. You are certainly one of the most passionate scientists who I would look up to! Thank you for being supportive and giving me this important opportunity in my academic life. Thank you for making all my field trips in Tanzania possible, easy and productive. Thank you for being helpful whenever I come at the University of Dar es salaam. I am so thankful for all the times you followed me in field trips in Tanzania. Thank you so much for all the advises in professional and personal life all the times. You have such a motherly heart. Thank you for all the encouragement, availability, advices and support whenever I needed . I am so grateful for all the support and encouragement when I lost my father few months before I finish my PhD. Thank you for the first trip in Uppsala for workshop which introduced me to Sanja.

Sanja Tibell; I was lucky enough to be your student when I barely knew what is phylogeny or lichen. You taught me many things, from scientific knowledge to how to be a qualified scientist. Your inspiration, availability, endless attention, patience, support and encouragement have always given me confidence, whenever I almost lost it. Thanks for teaching me DNA isolations, PCR amplifications and alignment preparation. Thank you giving me freedom to work on my ideas and to be challenged. There should me more people like you in the academics. Thank you for allowing me to call any time when I am having some questions even late nights as I was to submit my thesis. I am so much grateful for the time that you had to come to job on weekends for my research. Huge thanks for following me to Tanzania for the fieldtrip that means almost everything in my PhD studies. I have learnt a lot from your patience in many ways, thank you for being good advisor on professional and personal matters, for giving me freedom to discuss with you many life aspects it was real relaxing. Thank you for warmly welcoming me at Uppsala that I almost didn't feel the culture shock. Thank you for all the toys for my son, Giovanni and all the *Julbord* lunches at Ikea. I have a lot to thank you Sanja! I owe my sincerest gratitude for you.

I am deeply indebted to **Leif Tibell**; for the endless support, attention, patience and availability throughout my PhD studies. I feel very lucky to get the

opportunity to benefit from your wide lichenological knowledge in numerous fruitful discussions on the history of lichenology, lichen nomenclature and taxonomy. It was very fun discussing all the lichen aspects with you. I am extremely amazed on your continuous passion to work, discuss and teach about lichens. Thank you for teaching me about calicioid lichens, I couldn't imagine how cool they are until I learn from you. Many thanks for building such a strong foundation and stimulating my interest on lichens. If at all I can say today I like and enjoy research on lichens and yes, I do, I surely got the passion from you. Despite your long time experience you allowed me to express my ideas and let them be. I have benefited a lot from your passion on history of lichenology and making things interesting even when they are difficult, this I will always take with me. Thank you for the amazing field trips in Uppsala and the major one in Tanzania. You taught me how stubborn I have to be in the field to have good collection. Thank you for correcting my English and let my text be. Thank you for editing my summary in Swedish. Thank you for all the Swedish dishes you offered especially the fermented fishes, it was fun experience. I have learnt a lot from you. *Tack så mycket!*

I would like to extend my thanks to **Philippe Clerk**; for teaching me how to deal with *Usnea*. Thank you for teaching me to recognise the species of *Usnea* and to identify chemical compounds. I have learnt a lot from working with you on the microscope seeing different characters useful for *Usnea* and fruitful discussions. Many thanks for hosting me in your house, a week that I spent in Geneva at the Conservatoire et Jardin botanique of the City of Geneva (CJBG) for the TLC analyses. I owe so much gratitude to your family, Yama-mama and Linda for their warm welcome and kindness.

Many thanks to **Göran Thor** for I accepting me to follow you to the field trip in Gotland in the first year of my studies. I have to admit, it was my very first longest field trip on lichens. I learnt a lot. Thank you for all the discussions on my work. Thank you for reading my works and giving me inputs. Thank you for translating my summary in Swedish. I owe so much thanks to you for your availability whenever I needed support.

I would like to thank **Hanna Johansson**; for all the nice discussions on lichens, suggestions on my work and availability on my PhD in general. Thank you for all the meetings to discuss progress of my work. Thank you for all the positive compliments after my presentations. It was truly lifting.

My sincere gratitude to **Ulf** and **Kristina** at Pharmacognosy unit at Biomedical central for offering me good research environment and support when I was doing my experiments on chemical compounds. I hope I can work on that in the near future. Thank you very much!

My gratitude is extended to **Prof. Anthony Mshandete** at The Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania for opening my eyes to the academic opportunities. Thank you so much for all the advises that have huge impact on my carrier. I can recall, I heard the word 'lichens' for the first time from you. Thank you for all the

support availability and following my progress despite your busy schedule. Thank you for all the support and encouragement when I lost my father few months before I finish my PhD. Looking at my achievement today I owe so much gratitude to you.

Thank you very much **Prof. Amelia Kivaisi** at University of Dar es salaam, Tanzania for being always supportive and for all the advises and encouragements.

I would like to thank my colleagues and members of Systematic Biology program, **Ioana**; thank you for all the discussions about lichens and for all the help you provided whenever I needed. I am very impressed with your continuous enthusiasm on lichens. It was very relaxing whenever we shared stories about everything. It was always fun to talk to you. **Lorena**; thank you for the good moments we shared whenever we met, I love the positive energy in you, thank you for speaking up about *Usnea*.... being one of the few PhD students on lichen at the department it was always good to discuss with you about lichens. **Jane**... thank you very much for the big smile you always wear, it's so enriching! Thank you for all the discussions on research, life and fun things. I really appreciate all the positive energy and support from you. **Juma, Raquel, Julia, Mahwash, Marcus, Anders, Sara, Diem, Faheema, Anneli, Hendrik, Fawzeia, Jasper, Brendan, Sanea, Valentina, Shadi and Aron**; thank you for being my colleague and friends and for all the support. I appreciate. Thanks to **Martin, Sandie, Magnus, Fabien, Mats, Inger, Mikael and Petra**.

I would like to thank **Nahid**, for being helpful. You have made lab a pleasant place to work for your patience, ability to keep everything in the lab in order and your availability whenever needed, you have been of great help!

My thanks to Uppsala Herbarium members, **Martin, Mats, Monica, Jan, Stefan**, and others; you have been great help whenever I show up during my studies.

Great thank to the staffs at **Department of molecular biology and biotechnology University of Dar es salaam, Tanzania** for being always supportive whenever I come to Tanzania. Thank to Mr. Frank Mbago for the help in identifying host plants during my field trips in Tanzania. My sincere thanks goes to **Aneth David** for the kindness, friendship and support always. Thank you very much **Winnie** and **Kelvin** for the amazing time that we spent in Uppsala and all the support. It was short time but It was very fun working and exploring Uppsala together. Winnie '*Vini*' you are such a wonderful big sister since I know you.....thank you for everything!

I owe so much gratitude for the Prof. Tibuhwa student's group at The University of Dar es Salaam; **Zuhura, Willium, Stanslaus, Nana, Anthonia and Juma**; thank you for your kindness and support.

My big thanks go to the Tanzanians in Sweden; **Maglan, Donath, Lwidiko, Watson, Jasphet, Peter, Ally, Merezia, Bernadeta, Alice, Grace**,

Happy, Juma and others. Thank you for all the refreshing home stories, parties and cooking of Tanzanian dishes together. It was always fun to hang out with you.

My sincere thanks are extended to my family; I would like to express my profoundest gratitude to my dear husband, **Honest**; thank you for being a loving, patient, understanding, caring and supportive partner to me. Thank you for allowing me travel to Sweden for my PhD studies few months after our wedding. I am extremely grateful for listening to all of my complains with open heart when I was coping with the Swedish winter and lifestyles. Thanks for preparing all the envelopes for collecting my lichen samples. Thanks for visiting me in Uppsala. Thanks for taking care of our home when I am away and for assuring that our son is happy always. Thanks for all the prayers and encouragements. I love you so much!

Many thanks to my son, **Giovanni**; I am very blessed to have you in the first year of my PhD studies. You are my inspiration to achieve greatness. I know you were very young but you kindly agree stay with your grandparents/my parents with so much love. The bond you have with them is imaginable but you love me in a very special way! Thanks for blessing me with powerful smile on video call every morning to start my day and evening to refresh my ideas. I know I have set good example for you in the future. I love you!

My profound gratitude goes to my beloved, parents, **Mr Gilbert Temu** and **Mrs Aurelia Temu**; for all the love, encouragement and prayers they have sent along this journey. Thank you for trusting and being always supportive to me. I am extremely thanking you very much for taking care of my son while I am in Sweden. Your unconditional love and support has meant the world to me. Thank you for calling me every day to see if I am okay. I know that I have made you proud. I am blessed to have you. *Baba...* I know you couldn't wait for me to finish my studies, I hardly believe the fact that you died very few months before I defend my PhD. I know exactly how excited you were for my graduation. You were always proud of me, *asante sana! Mama... asante sana, ninakuombea na ninakupenda sana!*

I would like to thank my siblings, **Janeth, Herbert** and **Godson**; thank you for everything. You made me stronger and strengthen my spirit to succeed.

My gratitude are extended to my brother in law **Polycarp**; thank you for editing the cover of my thesis. I appreciate so much your patience to listen to my wishes, you know how many times you had to change until it come out as I needed. Thanks for all IT terminologies that you had to teach me. Thank you very much!

I am greatly grateful for the Swedish International Development Cooperation Agency – UDSM-SIDA, Project No. 2221 for full time scholarship of my PhD studies.

Above all, I thank God almighty for giving the strength and perseverance to carry out this study successfully. Glory to God!

References

- Acharius, E. (1815). Afhandling om de cryptogamiske Vexter, som komma under namn av Calicioidea, Första Stycket. *Kungliga Svenska Vetenskapsakademiens Handlinger* 1815: 246–271.
- Acharius, E. (1816). Afhandling om de cryptogamiske Vexter, som komma under namn av Calicioidea, Andra Stycket. *Kungliga Svenska Vetenskapsakademiens Handlinger* 1816: 106–125: 260–291.
- Acharius, E. (1817). Afhandling om de cryptogamiske Vexter, som komma under namn av Calicioidea, Tredje Stycket. *Kungliga Svenska Vetenskapsakademiens Handlinger* 1817: 220–244.
- Alstrup, V. & Aptroot, A. (2005). Pyrenocarpous lichens from Tanzania and Kenya. *Cryptogamie, Mycologie* 26: 265–271.
- Alstrup, V. & Christensen, S. (2006). New records of lichens with cyanobacteria from Tanzania and Kenya. *Cryptogamie, Mycologie* 27: 57–68.
- Alstrup, V., Aptroot, A., Divakar, P. K., LaGreca, S. & Tibell, L. (2010). Lichens from Tanzania and Kenya III: Macrolichens and calicioid lichens. *Cryptogamie Mycologie* 31: 333–351.
- Articus, K. (2004). *Phylogenetic studies in Usnea (Parmeliaceae) and allied genera* (Doctoral dissertation, Acta Universitatis Upsaliensis).
- Aschenbrenner, I.A., Cardinale, M., Berg, G. & Grube, M. (2014). Microbial cargo: do bacteria on symbiotic propagules reinforce the microbiome of lichens? *Environmental microbiology* 16(12): 3743–3752.
- Awasthi, G (1986): Lichen genus *Usnea* in India. *Journal of the Hattori Botanical Laboratory* 61: 333–421.
- Bates, S.T., Donna, B.L., Lauber, C.L., Walters, W.A., Knight, R. & Fierer, N. (2012). A preliminary survey of lichen associated eukaryotes using pyrosequencing. *The Lichenologist* 44(1): 137–146.
- Bessey, C.E. (1907) A Synopsis of Plant Phyla; University of Nebraska Studies: Lincoln, NE, USA, Vol. 7, pp. 275–373.
- Benítez, A., Prieto, M., González, Y. & Aragón, G. (2012). Effects of tropical montane forest disturbance on epiphytic macrolichens. *Science of the Total Environment* 441: 169–175.
- Blanco, O., Crespo, A., Ree, R.H. & Lumbsch, H.T. (2006). Major clades of parmelioid lichens (Parmeliaceae, Ascomycota) and the evolution of their morphological and chemical chemical diversity. *Molecular Phylogenetics and Evolution* 39: 52–69.
- Bryson, B. & Matthews, R. (2003). *A short history of nearly everything* (Vol. 258). New York: Broadway Books.
- Bubb, P., May, I., Miles, L. & Sayer, J. (2004). *Cloud Forest Agenda*. Unite Nations Environmental Programme, Cambridge, UK. 32pp.
- Cansaran, D., Kahya, D., Yurdakulol, E. & Atakol, O. (2006). Identification and quantitation of usnic acid from the lichen *Usnea* species of Anatolia and antimicrobial activity. *Zeitschrift für Naturforschung C* 61(11–12): 773–776.

- Caviró, E.A. (2015). Sistemática integrada del género *Usnea* Dill. Ex Adans. (Parmeliaceae) en la Península Ibérica. PhD dissertation. Madrid, Spain, 328 pp.
- Clements, F.F. & Shear, C.L. (1931). *The genera of fungi*. New York, The H.W. Wilson Company.
- Clerc, P. (1984). Contribution à la revision de la systématique des Usnées (Ascomycotina, *Usnea*) d'Europe. I. *Usnea florida* (L.) Wigg. emend. Clerc. *Cryptogamie - bryologie et lichénologie* 5: 333–360.
- Clerc, P. (1987a). Systematics of the *Usnea fragilescens* aggregate and its distribution in Scandinavia. *Nordic Journal of Botany* 7: 479–495.
- Clerc, P. (1987b). On the morphology of soralia in the genus *Usnea*. *Bibliotheca Lichenologica* 25: 99–102.
- Clerc, P. (1992). Some new or interesting species of the genus *Usnea* (lichenized Ascomycetes) in the British Isles. *Candollea* 47: 513–526.
- Clerc, P. (1994). Comment *Usnea mutabilis* Stirton, une espèce nord-américaine, se cache en Europe sous le nom d'*Usnea marocana* Motyka. Une contribution à la systématique du genre *Usnea* (Ascomycètes lichénisés). *Bulletin de la Société Linnéenne de Provence* 45: 309–316.
- Clerc, P. (1998). Species concepts in the genus *Usnea* (lichenized ascomycetes). *The Lichenologist* 30: 321–340.
- Clerc, P. (2006). Synopsis of *Usnea* (lichenized Ascomycetes) from the Azores with additional information on the species in Macaronesia. *The Lichenologist* 38: 191–212.
- Clerc, P. (2007). *Usnea*. In: Lichen Flora of the Greater Sonoran Desert Region Vol. 3 (T.H. Nash, III, C. Gries & F. Bungartz, eds.): 302–335. Tempe: Lichen Unlimited, Arizona State University.
- Clerc, P. (2011). *Usnea*. In Nordic Lichen Flora, Vol. 4 (A. Thell & R. Moberg, eds): 107–127. Uppsala: Nordic Lichen Society.
- Clerc, P. (2016). Notes on the genus *Usnea* (lichenized Ascomycota, Parmeliaceae). IV. *Herzogia* 29: 403–411.
- Crespo, A., Kauff, F., Divakar, P.K., del Prado, R., Pérez-Ortega, S., de Paz G.A., Ferencova, Z., Blanco, O., Roca-Valiente, B., Núñez-Zapata, J., Cubas, P., Argüello, A., Elix, J.A., Esslinger, T.L., Hawksworth, D.L., Millanes, A., Molina, M.C., Wedin, M., Ahti, T., Aptroot, A., Barreno, E., Bungartz, F., Calvelo, S., Candan, M., Cole, M., Ertz, D., Goffinet, B., Lindblom, L., Lücking, R., Lutzoni, F., Mattsson, J.E., Messuti, M.I., Miadlikowska, J., Piercey-Normore, M., Rico, V.J., Sipman, H.J.M., Schmitt, I., Spribille, T., Thell, A., Thor, G., Upreti, D.K. & Lumbsch, H.T. (2010). Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. *Taxon* 59: 1735–1753.
- Crespo, C., Lumbsch, H.T., Mattsson, J.E., Blanco, O., Divakar, P.K., Articus, K., Wiklund, E., Bawingan, P.A. & Wedin, M. (2007). Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear RPB1 gene. *Molecular Phylogenetics and Evolution* 44: 812–824.
- Culberson, C.F. & Ammann, K. (1979). Standardmethode zur Dünnschichtchromatographie von Flechtensubstanzen. *Herzogia* 5: 1–24.
- Culberson, C.F. & Johnson, A. (1982). Substitution of methyl tert-butyl ether for diethyl ether in the standardized thin-layer chromatographic method for lichen products. *Journal of Chromatography* 238: 483–487.
- Dillenius, J.J. (1742). *Historia Muscorum*. Oxford.

- Divakar, P.K., Crespo, A., Kraichak, E., Leavitt, S.D., Singh, G., Schmitt, I. & Lumbsch, H.T. (2017). Using a temporal phylogenetic method to harmonize family- and genus-level classification in the largest clade of lichen-forming fungi. *Fungal Diversity* 84: 101–117.
- Divakar, P.K., Crespo, A., Wedin, M., Leavitt, S.D., Hawksworth, D.L., Myllys, L., McCune, B., Randle, T., Bjerke, J.W., Ohmura, Y., Schmitt, I., Boluda, C.G., Alors, D., Roca-Valiente, B., Del-Prado, R., Ruibal, C., Buaruang, K., Núñez-Zapata, J., Amo de Paz, G., Rico, V.J., Molina, M.C., Elix, J.A., Esslinger, T.L., Tronstad, I.K.K., Lindgren, H., Ertz, D., Gueidan, C., Saag, L., Mark, K., Singh, K., Dal Grande, F., Parmen, S., Beck, A., Benatti, M.N., Blanchon, D.J., Candan, M., Clerc, P., Goward, T., Grube, M., Hodgkinson, B.P., Hur, J.S., Kantvilas, G., Kirika, P.M., Lendemer, J., Mattsson, J.E., Messuti, M.I., Miadlikowska, J., Nelsen, M., Ohlson, J.I., Pérez-Ortega, S., Saag, A., Sipman, H.J.M., Sohrabi, M., Thell, A., Thor, G., Truong, C., Yahr, R., Upreti, D.K., Cubas, P. & Lumbsch, H.T. (2015). Evolution of complex symbiotic relationships in a morphologically derived family of lichen-forming fungi. *New Phytologist* 208: 1217–1226.
- Dodge, C.W. (1956). Some lichens of tropical Africa. II. *Usnea*. *Annals of the Missouri Botanical Garden* 43: 381–396.
- Dodge, C.W. (1957). Some lichens of tropical Africa. II. *Usnea* (continued). *Annals of the Missouri Botanical Garden* 44: 1–76.
- FAO (2010a). *Global Forest Resources Assessment 2010 Country Report*. Food and Agriculture Organization, Rome, Italy. 56pp.
- FAO (2010b). *Global Forest Resources Assessment. 2010 Main Report*. Food and Agriculture Organization, Rome, Italy. 340pp.
- Fos, S. & Clerc, P. (2000). The lichen genus *Usnea* on *Quercus suber* in Iberian cork-oak forests. *The Lichenologist* 32: 67–88.
- Fries, E. (1831). *Lichenographia Europaea reformata. Praemittuntur lichenologiae fundamenta... Conscripsit Elias Fries,...* Typis Berlingianis.
- Fries, T. M. (1860). *Lichenes arctoi Europae Groenlandiaeque hactenus cogniti* (Vol. 3). Leffler.
- Fries, T. M. (1861). *Miscellanea lichenologica. Flora* 44: 409–413.
- Galloway, D. (2007). *Flora of New Zealand Lichens*. Revised Second Edition Including Lichen-Forming and Lichenicolous Fungi. Volumes 1 and 2. Manaaki Whenua Press, Lincoln, New Zealand. 2261 pp.
- Gardes, M. & Bruns, T.D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular ecology* 2:113–118.
- Gibson, L., Lee, T. M., Koh, L. P., Brook, B. W., Gardner, T. A., Barlow, J., Peres, C. A., Bradshaw, C. J. A., Laurance, W. F., Lovejoy, T. E. & Sodhi, N. S. (2011). Primary forests are irreplaceable for sustaining tropical biodiversity. *Nature* 478: 378 – 381.
- Gerlach, A.D.C.L., Toprak, Z., Naciri, Y., Caviro, E. A., da Silveira, R. M. B., & Clerc, P. (2019). New insights into the *Usnea cornuta* aggregate (Parmeliaceae, lichenized Ascomycota): Molecular analysis reveals high genetic diversity correlated with chemistry. *Molecular phylogenetics and evolution* 131: 125–137.
- Grube, M., Cernava, T., Soh, J., Fuchs, S., Aschenbrenner, I., Lassek, C., Wegner, U., Becher, D., Riedel, K., Sensen, C.W. & Berg, G. (2015). Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. *The ISME journal* 9: 412–424.
- Halonen, P. (2000). *Studies on the lichen genus Usnea in East Fennoscandia and Pacific North America*. PhD thesis. Oulu: University of Oulu.

- Halonen, P., Myllys, L., Ahti, T. & Petrova, O.V. (1999). The lichen genus *Usnea* in East Fennoscandia. III. The shrubby species. *Annales Botanici Fennici* 36: 235–256.
- Halonen, P., Clerc, P., Goward, T., Brodo, IM, & Wulff, K. (1998). Synopsis of the genus *Usnea* (lichenized ascomycetes) in British Columbia, Canada. *The Bryologist* 36–60.
- Hemp, A. (2006a). Continuum or zonation? Altitudinal gradients in the forest vegetation of Mt. Kilimanjaro. *Plant ecology* 184: 27–42.
- Hemp, A. (2006b). Vegetation of Kilimanjaro: Hidden endemics and missing bamboo. *African Journal of Ecology* 44: 305–328.
- Herrera-Campos, M.A. (2016). *Usnea* in Mexico. *Bibliotheca Lichenologica* 110: 505–620.
- Herrera-Campos, M.A., Nash, T.H., III & Garcia, A.Z. (2001). Preliminary study of the *Usnea fragilesceus* aggregate in Mexico. *The Bryologist* 104: 235–259.
- Herrera-Campos, M. A., Clerc, P., & Nash III, T. H. (1998). Pendulous species of *Usnea* from the temperate forests in Mexico. *The Bryologist* 303–329.
- Hillis, D.M. & Bull, J.J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic biology* 42: 182–192.
- Hinds, J.W. & Hinds, P.L. (2007). The Macrolichens of New England. In: *Memoirs of the New York Botanical Garden* No. 96. New York: New York Botanical Garden Press, Bronx, 584 pp.
- Honegger, R. (1998). The lichen symbiosis—what is so spectacular about it? *The Lichenologist* 30: 193–212.
- Insarov, G.E., Semenov, S.M. & Insarova, I.D. (1999). A system to monitor climate change with epilithic lichens. *Environmental Monitoring and Assessment* 55(2): 279–298.
- Kaasalainen, U., Hemp, A. & Rikkinen, J. (2018). Lichen diversity on Mt. Kilimanjaro. In C. Hemp, K. Böhning-Gaese, M. Fischer, & A. Hemp (Eds.), *The KiLi Project: Kilimanjaro ecosystems under global change: Linking biodiversity, biotic interactions and biogeochemical ecosystem processes* (pp. 74–75). Senckenberg Gesellschaft für Naturforschung.
- Keissler, K.V. (1938). Pyrenulaceae, Mycoporaceae, Coniocarpineae. In *Kryptogamen-Flora von Deutschland, Österreich und der Schweiz*; Rabenhorst, G.L., Ed.; Akademische Verlagsgesellschaft: Leipzig, Germany, pp. 1–846.
- Krog, H. (1993). *Parmelina enormis* (Hale) Hale is *Bulbothrix enormis* (Hale) Krog comb. nov. *The Lichenologist* 25: 299–300.
- Krog, H. (1994). New observations on *Usnea* subgenus *Eumitria* in eastern and central Africa. In: *Proceedings of the XIII plenary meeting AETFAT Vol. 2* (J. Seyani & A. Chikuni, eds.): 813–821. Malawi: National Herbarium and Botanic Gardens.
- Krog, H. (2000). *Corticolous macrolichens of low montane rainforests and moist woodlands of eastern Tanzania*. Botanical Garden and Museum-University of Oslo.
- Krog, H. & Swinscow, T.D.V. (1982). A First Report of *Parietina* in the Genus *Parmelia* s. lat. *The Lichenologist* 14: 98–98.
- Krog, H. & Swinscow, T.D.V. (1983). A new species and new combinations in *Parmotrema* (Parmeliaceae). *The Lichenologist* 15: 127–130.
- Krog, H. & Swinscow, T.D.V. (1986a). The lichen genera *Lasallia* and *Umbilicaria* in east Africa. *Nordic Journal of Botany* 6: 75–85.
- Krog, H. & Swinscow, T.D.V. (1986b). *Parmotrema hicksii* sp. nov. described from Tanzania. *The Lichenologist* 18: 292–293.

- Krog, H., Seyani, J. & Chikuni, A. (1994). New observations on *Usnea* subgenus *Eumitria* in eastern and central Africa. In Proceedings of XIIIth Plenary Meeting AETFAT, Malawi (pp. 813–821).
- Kruys, N. & Jonsson, B.G. (1997). Insular patterns of calicioid lichens in a boreal old-growth forest-wetland mosaic. *Ecography* 20: 605–613.
- Lambrechts, C., Woodley, B., Hemp, A., Hemp, A. & Nnyiti, P. (2002). *Aerial Survey of the Threats to Mt. Kilimanjaro Forests*. UNDP, Dar es Salaam. 29pp.
- Larsson, A. (2014). AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30: 3276–3278.
- Lücking, R., Hodkinson, B.P. & Leavitt, S.D. (2017). The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota—Approaching one thousand genera. *The Bryologist* 119: 361–417.
- Lumbsch, H.T. & Wirtz, N. (2011). Phylogenetic relationships of the neuropogonoid core group in the genus *Usnea* (Ascomycota: Parmeliaceae). *The Lichenologist* 43: 553–559.
- Matheny, P.B., Liu, Y.J., Ammirati, J.F. & Hall, B.D. (2002). Using RPB1 sequences to improve phylogenetic inference among mushrooms (*Inocybe*, Agaricales). *American Journal of Botany* 89: 688–698.
- Motyka, J. (1936). *Lichenum generis Usnea studium monographicum* (No. 1). Leopoli.
- Motyka, J. (1938). *Lichenum generis Usnea studium monographicum*. Pars systematica (Vol. 2). Leopoldi (privately printed).
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A. & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature* 403(6772), 853.
- Nitare, J. (2000). *Signalarter: indikatorer på skyddsvärd skog: flora över kryptogamer* / Skogsstyrelsen förlag: Jönköping. In Swedish 384 pp.
- Ohmura, Y. (2001). Taxonomic study of the genus *Usnea* (lichenized Ascomycetes) in Japan and Taiwan. *Journal of the Hattori Botanical Laboratory* 90: 1–96.
- Ohmura, Y. (2002). Phylogenetic evaluation of infrageneric groups of the genus *Usnea* based on ITS regions in rDNA. *Journal of the Hattori Botanical Laboratory* 92: 231–243.
- Ohmura, Y. (2008). Taxonomy and molecular phylogeny of *Usnea rubicunda* and *U. rubrotincta* (Parmeliaceae, lichenized Ascomycotina). *Journal of Japanese Botany* 83: 347–355.
- Ohmura, Y. (2012). A synopsis of the lichen genus *Usnea* (Parmeliaceae, Ascomycota) in Taiwan. *Memoirs of the National Museum of Nature and Science* 48: 91–137.
- Ohmura, Y. & Kanda, H. (2004). Taxonomic status of section *Neuropogon* in the genus *Usnea* elucidated by morphological comparisons and ITS rDNA sequences. *The Lichenologist* 36: 217–225.
- Ohmura, Y., Skirina, I. & Skirin, F. (2017). Contribution to the knowledge of the genus *Usnea* (Parmeliaceae, Ascomycota) in southern far East Russia. *Bulletin of the National Museum of Nature and Science, series B* 43: 1–10.
- Podterob, A. P. (2008). Chemical composition of lichens and their medical applications. *Pharmaceutical Chemistry Journal* 42: 582–588.
- Posada, D. & Crandall, K.A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- Prieto, M., Baloch, E., Tehler, A. & Wedin, M. (2013). Mazaedium evolution in the Ascomycota (Fungi) and the classification of mazaediate groups of unclear relationship. *Cladistics* 29: 1–13.
- Prieto, M. & Wedin, M. (2017). Phylogeny, taxonomy and diversification events in the Caliciaceae. *Fungal Diversity* 82: 221–238.

- Rambaut, A. & Drummond, A.J. (2010). *FigTree v1. 3.1*. Institute of Evolutionary Biology; University of Edinburgh: Edinburgh, UK.
- Rodriguez, J.M. (2011). *The Gene Usnea (Liquid Ascomycetes) in Argentina: Systematic and Biogeographic Study* (Doctoral dissertation, Thesis, Faculty of Exact Sciences, Physics and Natural Sciences, National University of Córdoba).
- Rodriguez, J.M., Estrabou, C., Truong, C. & Clerc, P. (2011). The saxicolous species of the genus *Usnea* subgenus *Usnea* (Parmeliaceae) in Argentina and Uruguay. *The Bryologist* 114: 504–525.
- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S. & Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* 61: 539–542.
- Saag, L., Torra, T., Saag, A., Del-Prado, R. & Randlane, T. (2011). Phylogenetic relations of European shrubby taxa of the genus *Usnea*. *The Lichenologist* 43: 427–444.
- Schmitt, I., Crespo A., Divakar P.K., Fankhauser J.D., Herman-Sackett, E., Kalb, K. & Widhalm, T. (2009). New primers for promising single-copy genes in fungal phylogenetics and systematics. *Persoonia* 23: 35–40.
- Seymour, F.A., Crittenden, P.D. & Dyer, P.S. (2005). Sex in the extremes: lichen-forming fungi. *Mycologist* 19: 51–58.
- SLU Artdatabanken (2020). Rödlistade arter i Sverige 2020. SLU, Uppsala (in Swedish).
- Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M.C. & Mayrhofer, H. (2016). Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353: 488–492.
- Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- Stevens, G.N. (1992). Variation in thallus morphology in response to climatic and geographical distribution in an *Usnea* complex. *The Lichenologist* 24: 229–248.
- Stevens, G.N. (1999). A revision of the lichen family Usneaceae in Australia. *Bibliotheca Lichenologica* 72: 1–128.
- Stevens, G.N. (2004). Usneaceae. In: Flora of Australia Vol. 56A, Lichens 4 (P.M. McCarthy & K. Mallett, eds.): 78–98 & 107–115. Melbourne: ABR/CSIRO.
- Stirton, J. (1882). Notes on the genus *Usnea* with description of new species. *Scottish Naturalist* 6: 292–297.
- Swinscow, T.D.V. & Krog, H. (1974). *Usnea* subgenus *Eumitria* in East Africa. *Norwegian Journal of Botany* 21: 165–185.
- Swinscow, T.D.V. & Krog, H. (1975). The *Usnea undulata* aggregate in East Africa. *The Lichenologist* 7: 121–138.
- Swinscow, T.D.V. & Krog, H. (1976a). The *Usnea articulata* aggregate in East Africa. *Norwegian Journal of Botany* 23: 261–268.
- Swinscow, T.D.V. & Krog, H. (1976b). The *Usnea bornmuelleri* aggregate in East Africa. *Norwegian Journal of Botany* 23: 23–31.
- Swinscow, T.D.V. & Krog, H. (1978). Pendulous species of *Usnea* in East Africa. *Norwegian Journal of Botany* 25: 221–241.
- Swinscow, T.D.V. & Krog, H. (1979). The fruticose species of *Usnea* subgenus *Usnea* in East Africa. *The Lichenologist* 11: 207–252.
- Swinscow, T.D.V. & Krog, H. (1986a). *Usnea antiqua* sp. nov. described from Tanzania. *The Lichenologist* 18: 293–295.
- Swinscow, T.D.V. & Krog, H. (1986b). Some observations on the thallus in *Pannaria*, with description of a new species. *The Lichenologist* 18: 309–315.

- Swinscow, T.D.V. & Krog, H. (1986c). A new species in the genus *Collema* from East Africa. *The Lichenologist* 18: 63–70.
- Swinscow, T.D.V. & Krog, H. (1988). *Macrolichens of East Africa*. British Museum (Natural History). London, U.K.
- Tavares, I.I. & Sanders, W.B. (1998). Preliminary report on the short, apotheciate taxa of *Usnea* in the southwestern United States. In: Lichenographia Thomsoniana: North American Lichenology in Honor of John W. Thomson (M. G. Glenn, R. C. Harris, R. Dirig & M. S. Cole, eds): 171–185. Ithaca, New York: Mycotaxon Ltd.
- Temu, S.G., Clerc, P., Tibell, L., Tibuhwa, D.D. & Tibell, S. (2019a). Phylogeny of the subgenus *Eumitria* in Tanzania. *Mycology* 10: 250–260.
- Temu, S.G., Tibell, S., Tibuhwa, D.D. & Tibell, L. (2019b). Crustose Calicioid Lichens and Fungi in Mountain Cloud Forests of Tanzania. *Microorganisms* 7: 491.
- Thell, A., Crespo, A., Divakar, P.K., Kärnefelt, I., Leavitt, S.D., Lumbsch, H.T. & Seaward, M.R. (2012). A review of the lichen family Parmeliaceae—history, phylogeny and current taxonomy. *Nordic Journal of Botany* 30: 641–664.
- Thell, A., Kärnefelt, I. & Seaward, M.R. (2018). Splitting or synonymizing—genus concept and taxonomy exemplified by the Parmeliaceae in the Nordic region. *Graphis Scripta* 30: 130–137.
- Tibell, L. (1980). The lichen genus *Chaenotheca* in the Northern Hemisphere. *Symbolae Botanicae Upsaliensis* 23(1): 1–65.
- Tibell, L. (1984). A reappraisal of the taxonomy of Caliciales. *Nova Hedwigia Beiheft* 79: 597–713.
- Tibell, L. (1987). Australasian Caliciales. *Symbolae Botanicae Upsalienses* 27(1): 1–279.
- Tibell, L. (1992). Crustose lichens as indicators of forest continuity in boreal coniferous forests. *Nordic Journal of Botany* 12: 427–450.
- Tibell, L. (1996). *Caliciales*. Flora Neotropica, 69, New York Botanical Garden, New York.
- Tibell, L. (1998). Crustose mazaediate lichens and the Mycocaliciaceae in temperate South America. *Bibliotheca Lichenologica* 71: 5–107.
- Tibell, L. (2001). A synopsis of crustose calicioid lichens and fungi from mainland Africa and Madagascar. *Nordic Journal of Botany* 20: 717–742.
- Tibell, L. (2003). *Tholurna dissimilis* and generic delimitations in Caliciaceae inferred from nuclear ITS and LSU rDNA phylogenies (Lecanorales, lichenized ascomycetes). *Mycological research* 107: 1403–1418.
- Tibell, L. (2006). Calicium in the Indian Himalayas. *Journal of the Hattori Botanical Laboratory* 100: 809–851.
- Tibell, L. & Thor, G. (2003). Calicioid lichens and fungi of Japan. *Journal of the Hattori Botanical Laboratory* 94: 205–259.
- Tibell, L. & Wedin, M. (2000). Mycocaliciales, a new order for nonlichenized calicioid fungi. *Mycologia* 577–581.
- Tibell, L., Tibell, S. & Van Der Pluijm, A. (2019). *Chaenotheca biesboschii* a new calicioid lichen from willow forests in the Netherlands. *The Lichenologist* 51: 123–135.
- Titov, A. (2000). Notes on calicioid lichens and fungi from the Gongga Mountains (Sichuan, China). *The Lichenologist* 32: 553–569.
- Titov, A. (2001). Further notes on calicioid lichens and fungi from the Gongga Mountains (Sichuan, China). *The Lichenologist* 33: 303–314.
- Truong, C. (2012). *Systematics of the lichen genus Usnea in tropical South America*. PhD dissertation. University of Geneva, Faculty of Sciences. 286 pp.

- Truong, C. & Clerc, P. (2012). The lichen genus *Usnea* (Parmeliaceae) in tropical South America: species with a pigmented medulla, reacting C + yellow. *The Lichenologist* 44: 625–637.
- Truong, C., Divakar, P.K., Yahr, R., Crespo, A. & Clerc, P. (2013a). Testing the use of ITS rDNA and protein-coding genes in the generic and species delimitation of the lichen genus *Usnea* (Parmeliaceae, Ascomycota). *Molecular Phylogenetics and Evolution* 68: 357–372.
- Truong, C. & Clerc, P. (2013). Eumitrioid *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. *The Lichenologist* 45: 383–395.
- Truong, C. & Clerc, P. (2016). New species and new records in the genus *Usnea* (Parmeliaceae, lichenized Ascomycota) from tropical South America. *The Lichenologist* 48: 71–93.
- Truong, C., Bungartz, F. & Clerc, P. (2011). The lichen genus *Usnea* (Parmeliaceae) in the tropical Andes and the Galapagos: species with a red–orange cortical or subcortical pigmentation. *The Bryologist* 114: 477–503.
- Truong, C., Rodriguez, J.M. & Clerc, P. (2013b). Pendulous *Usnea* species (Parmeliaceae, lichenized Ascomycota) in tropical South America and the Galapagos. *The Lichenologist* 45: 505–542.
- Tuovinen, V., Ekman S., Thor, G., Vanderpool, D., Spribille, T. & Johannesson, H. (2019). Two basidiomycete fungi in the cortex of wolf lichens. *Current Biology* 29: R88–R90.
- Vaidya, G., Lohman, D. J. & Meier, R. (2011). SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180.
- Vězda, A. (2008): Vězda: Lichenes selecti exsiccati (1960–1991). Alphabetical index to all 2500 edited taxa of lichenized or lichenicolous fungi. *Sauteria* 15: 571–596.
- Vilgalys, R. & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of bacteriology* 172: 4238–4246.
- Wagner, S. & Lugazo, S. (2011). *An Analysis of Key Stakeholders in the West Usambara Mountains of Korogwe and Lushoto Districts: Improving Livelihood Security and Sustainability for Rural Communities in the Eastern Arc Mountains Project*. Tanzania Forest Conservation Group. Tanzania Forest Conservation Group. <https://www.tfcg.org/wp-content/uploads/2018/05/West-Usambara-Stakeholder-Analysis-Report-2011-FINAL.pdf>
- Walker, F.J. (1985). The lichen genus *Usnea* subgenus *Neuropogon*. *Bulletin of the British Museum (Natural History), Botany* 13: 1–130.
- Wedin, M., Maier, S., Fernandez-Brime, S., Cronholm, B., Westberg, M. & Grube, M. (2016). Microbiome change by symbiotic invasion in lichens. *Environmental microbiology* 18: 1428–1439.
- White, T.J., Bruns, T.D., Lee, S. & Taylor, J.L. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols: a guide to methods and applications (M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White, eds): 315–322. Academic Press, San Diego.
- Wirtz, N., Printzen, C. & Lumbsch, H.T. (2008). The delimitation of Antarctic and bipolar species of neuropogonoid *Usnea* (Ascomycota, Lecanorales): A cohesion approach of species recognition for the *Usnea perpusilla* complex. *Mycological Research* 112: 472–484.

- Wirtz, N., Printzen, C. & Lumbsch, H.T. (2012). Using haplotype networks, estimation of gene flow and phenotypic characters to understand species delimitation in fungi of a predominantly Antarctic *Usnea* group (Ascomycota, Parmeliaceae). *Organisms Diversity & Evolution* 12: 17–37.
- Wirtz, N., Printzen, C., Sancho, L.G. & Lumbsch, T.H. (2006). The phylogeny and classification of *Neuropogon* and *Usnea* (Parmeliaceae, Ascomycota) revisited. *Taxon* 55: 367–376.
- Zahlbruckner, A. V. (1926). *Lichenes (Flechten)*. B. Spezieller Teil. Die natürlichen Pflanzenfamilien. Engelmann, Leipzig, 61–263.

Acta Universitatis Upsaliensis

*Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Science and Technology 1979*

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-422950



ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2020